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THE VALUE OF *IN VIVO* MONITORING AND CHLORINATION FOR THE CONTROL OF TOXIC CYANOBACTERIA IN DRINKING WATER PRODUCTION

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THE VALUE OF *IN VIVO* MONITORING AND CHLORINATION FOR THE CONTROL OF TOXIC CYANOBACTERIA IN DRINKING WATER PRODUCTION

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DEDICATION

For Lila!

برای مادرم، پدرم و البرز!

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RÉSUMÉ

La prolifération croissante des cyanobactéries (CBs) toxiques dans les sources d'eau potable et dans les usines de traitement d'eau potable est une préoccupation mondiale. L‟utilisation de sondes *in vivo*, permettant la détection de fluorescence de la phycocyanine (PC) des CBs, est une technologie émergente et largement employée pour détecter les CBs dans les eaux de source. Dans les traitements varies de l‟eau de source qui contient des CBs toxiques la chloration directe de cette eau peut engendrer l‟endommagement de cellules, causer le relargage de toxines ainsi que la formation des sous-produits de désinfection. La chloration, bien que prouvée être efficace pour l'oxydation de certaines espèces des CBs et des cyanotoxines, aucune information n‟est disponible sur sa cinétique de saxitoxines (STXS: saxitoxine, C-toxines et gonyautoxines) produites par *Anabaena circinalis*. De plus, il y a peu d'informations sur la chloration des différentes cellules toxiques de CBs, sur la demande en chlore des cellules de CBs, la chloration de toxines, et sur l‟éventualité de la formation de sous-produits de désinfection en présence de matériaux cellulaires. Par ailleurs, le sort des CBs, et les toxines qui leur sont associés à l'intérieur des usines de traitement d'eau potable (après différents procédés de traitement conventionnels et avancés) exigent une investigation plus approfondie. Les experts australiens sont les pionniers dans la gestion des problèmes reliés aux CBs toxiques. Dans le but d‟aider à la préparation d‟un plan de gestion des problèmes de CBs et leurs toxines pour la province du Québec (Canada), une visite industrielle a été financée pour documenter les expériences australiennes dans ce domaine. Cette thèse de doctorat comporte trois thèmes principaux qui seront développés dans ce document : (1) présence et surveillance (2) traitement et (3) gestion. L'objectif général de ce doctorat est d'étudier la surveillance *in vivo* de CB et de leurs toxines dans les sources d‟eau potable ainsi que leur élimination potentielle par traitement conventionnel et l'oxydation. Les objectifs spécifiques de ce projet de recherche sont les suivants : (1) valider l'utilisation de sondes de fluorescence *in vivo* basée sur des mesures de PC dans les eaux naturelles (2) étudier la distribution spatio-temporelle de CBs dans les masses d'eau susceptible d'avoir une prolifération de CBs au Québec, et de montrer l'impact de cette prolifération dans l'eau à la prise d'eau; (3) surveiller les concentrations de CBs et leurs toxines dans plusieurs prises d‟eau potable en utilisant des stratégies d'échantillonnage à haute fréquence et par événement (par exemple : échantillonner deux fois par semaine, l'échantillonnage lorsque le goût et l'odeur sont rapportés, etc.) avec une combinaison de dénombrement usuel et des méthodes en

ligne; (4) documenter l'efficacité du chlore à compromettre les différentes espèces de CBs, à oxyder une large gamme de cyanotoxines dans l'étape de prétraitement, et à réduire leurs potentiels toxiques. Déterminer et intégrer l'impact sur la qualité d'eau dans les modèles de prévision d'oxydation de cyanotoxines par le chlore; (5) étudier le relargage des matières organiques liées à la cellule, et aussi leur contribution aux précurseurs des sous-produits de désinfection lors de la chloration directe de cellules intactes; (6) analyser l'efficacité des stratégies de gestion de CBs appliqué par les différentes autorités d'eau en Australie pour empêcher la percée de CBs dans les usines de traitement d'eau potable; (7) pendant la formation de prolifération de CBs, détecter leurs potentielle toxiques et leurs toxines dans des échantillons d'eau en utilisant des kits ELISA et une méthode LC-MS/MS multitoxine, et (8) dans une usine de traitement à pilote et grande échelle, documenter l'enlèvement de CB et leurs toxines (intra - et extracellulaire) grâce à un traitement conventionnelle (coagulation, clarification et filtration) et d'oxydation (chlore).

La première section de cette thèse est d'étudier l'occurrence de CB et leurs toxines ainsi que leurs méthodes de surveillance. La performance des diverses sondes *in vivo* de cyanobactéries a été étudiée durant ce projet. Afin de comparer les lectures brutes des différentes sondes, une démarche novatrice a été utilisée pour présenter les lectures de sonde en biovolume équivalent de CBs. Deux causes d'interférence trouvée concernant la lecture de sonde sont la turbidité inorganique et la présence de la biomasse algale. Un facteur de correction avec les mesures simultanées de la chlorophylle a été développé pour les sondes. Les données de terrain attestent que la sous-estimation potentielle de la population de CBs est un enjeu majeur dans l'emploi des sondes *in vivo*. Surtout dans les cas où la sous-estimation de CBs correspond à des niveaux réels d'alerte utilisés pour déclencher des actions de surveillance et de gestion. En outre, l'intensive surveillance *in vivo* à la prise d'eau des usines a révélé des informations inédites sur l'impact des proliférations CBs dans ces prises d'eau.

La deuxième section de cette thèse étudie le traitement de la CBs et leurs toxines à l'aide d'oxydation avec le chlore. Lors de la chloration d'*Anabaena circinalis*, dont la valeur de chlore (CT) est à 7,0 mg.min/L, toutes les cellules ont perdu la viabilité provoquant le relargage de toxines. L'endommagement des membranes cellulaires d'*Anabaena circinalis* est survenu plus rapidement que l'oxydation des STXS libérés. Tous les saxitoxines ainsi que plus de 95% des autres analogues de STXS se sont ensuite oxydés. L'analyse cinétique de l'oxydation des

analogues de STXS révèle de considérables différences de sensibilité au chlore, la saxitoxine étant le plus facile à oxyder. De plus, les concentrations de trihalométhanes, des acides haloacétiques, et de la N-nitrosodiméthylamine (les sous-produits de la chloration) ont respectivement été de <50 µg/L et 11 ng/L, même à la plus haute valeur de CT (50,3 mg.min/L).

Les valeurs de CT de 172,4 et 100 mg.min / L ont été nécessaires lors de la chloration des cellules toxiques de *Microcystis aeruginosa* pour obtenir 76% lyse cellulaire puis une oxydation des toxines intracellulaires libérées inférieure à la valeur d'indication de 1 µg/L MC-L. . Pour les expériences de chloration dans l'eau ultrapure, les taux d'oxydation de toxine étaient semblables ou plus rapides que les taux de lyse cellulaire. Dans la plupart des cas concernant les expériences de chloration en eau naturelle, la lyse cellulaire est plus rapide que le taux d'oxydation des toxines. En outre, même avec une valeur maximale de CT à 3051,2 mg. min/L, les concentrations de trihalométhanes (12,87 µg/L) et les acides haloacétiques (sous la limite de détection) ont été inférieurs aux valeurs balises de chloration à 500000 cellules/mL de *Microcystis aeruginosa* en eau ultrapure.. Les cellules de souches toxiques d'*Anabaena circinalis*, de *Microcystis aeruginosa*, de *Cylindrospermopsis raciborskii* et d'une souche non toxique d'*Aphanizomenon issatsckenka* ont tous perdu viabilité avec une exposition au chlore (CT) de 4,0 mg.min/L 60% et ceci a provoqué le relargage de toxines (en cas de cellules toxiques). De plus, l'endommagement de membranes cellulaires survenues plus rapidement que l'oxydation des toxines libérées. L'analyse cinétique de l'oxydation des toxines en eau naturelle a révélé des différences considérables à leurs sensibilités au chlore, saxitoxines étant le plus facile à oxyder suivie par cylindrospermopsine et microcystine-LR. En plus, même à la plus haute valeur de CT (220 mg.min / L), les concentrations de trihalométhanes et des acides haloacétiques (<40 µg/L) et N-nitrosodiméthylamine (<10 ng/L), étant des sous-produits de la chloration, ont été à nouveau inférieurs à la valeur balise. Cependant, avec des nombres de cellules très élevés les concentrations de sous-produits de la chloration dans des conditions environnementales ont été au-dessus des valeurs indicatives.

Le dernier thème de cette thèse examine les défis de gestion du CB et leurs toxines envers la production de l'‟eau potable. Le sort des cellules de CBs et leurs toxines a été étudié parmi les étapes suivantes : après l'ajout de charbon actif en poudre, après la clarification, dans les boues du clarificateur, après filtration et postchloration dans une usine de traitement d'eau potable à pleine échelle, et ainsi dans le laboratoire que pour la chloration. L'accumulation de cellules de

CBs (maximum de 4,7 × 10^6 cellules/mL) et une concentration maximale cyanotoxines de 10 mg/L ont été détectées dans le processus de clarification. Une fois, 2,47 µg/L de microcystines ont été analysés dans eau potable et ceux-ci ont dépassé toutes les valeurs de balise recommandées par les autorités d‟eau à travers le monde. La chloration des échantillons prise directement de la prolifération environnementale de CBs n‟a pas reproduit l'élimination efficace des cellules et des toxines qui ont été antérieurement observées lors des essais en laboratoire utilisant des cellules cultivées et les toxines dissoutes standard.

Les six autorités/industries d'eau, qui ont été visitées pendant le séjour en Australie (mars-juillet 2010), fournissent de l'eau à plus de 72% de la population australienne ainsi que quatre grandes régions métropolitaines. Un questionnaire a été préparé et rempli afin de documenter les résultatsde ces visites. Dans ces états australiennes, les espèces principales formant des blooms de CBs sont *Microcystis aeruginosa* (produisant microcystines), *Anabaena circinalis* (produisant les saxitoxines), et plus récemment, *Cylindrospermopsis raciborskii* (produisant les cylindrospermopsine). Leurs plans de gestion comprennent la surveillance intensive de CBs non seulement dans les plans d'eau et la proximité des prises d'eau, mais aussi dans les affluents des réservoirs et des grands fleuves. L'emploi des sondes CB *in vivo* est également inclus dans leur plan de surveillance. La surveillance *in vivo* les aide à mieux comprendre la dynamique des CBs dans le réservoir ainsi que les limites des différentes techniques *in vivo*. Ces prélèvements fréquents de mesure *in vivo* leur fournissent un ensemble de données historiques extensif. Cette surveillance accrue leur a permis d'empêcher l'écoulement et l'accumulation des cellules toxiques de CBs dans des usines de traitement d'eau potable. Les autorités d'eau australiennes ont créé des balises alternatives dérivées de conseils nationaux et internationaux déjà en existence. De plus, ils ont mise en œuvre de balise site spécifique lié à la production d'eau potable, à la réutilisation d'eau et aux activités récréatives.

Cette thèse de doctorat se concentre sur la fourniture d'informations techniques pertinentes aux besoins de gestions de CBs pour l'industrie d'eau potable. Les résultats de laboratoire montrent qu'il est possible d'atteindre des objectifs contradictoires de qualité d'eau (oxydation toxine efficace tout en respectant les directives de sous-produit d'oxydation) en utilisant des conditions de chloration exercée dans une usine de traitement d'eau potable. Toutefois, dans des conditions opérationnelles, les opérateurs d'usine devront être prudents avec l'interprétation des résultats de laboratoire. Les autorités d'eau doivent accommoder les outils techniques et les seuils à des

conditions site-spécifiques pour pouvoir développer des outils d'intervention adaptés aux problèmes locaux. La documentation des problèmes locaux particuliers et des besoins site-spécifiques est le facteur clé dans cette procédure.

ABSTRACT

The increasing occurrence of toxic cyanobacterial blooms in drinking water (DW) sources and inside drinking water treatment plants (DWTPs) is a global concern. The applications of *in vivo* probes, which can detect the fluorescence of the cyanobacterial phycocyanin, are an emerging and widely used technology for cyanobacterial detection in source waters. Direct chlorination of source water containing toxic cyanobacterial cells for different treatment purposes might cause cell damage, toxin release and disinfection by-product (DBP) formation. While chlorination has been proven to be efficient for oxidation of certain cyanobacteria (CB) species and cyanotoxins, there is no information available on chlorination kinetics of saxitoxins (STXs: saxitoxin, C-toxins and, gonyautoxins) produced by *Anabaena circinalis*. Furthermore, there is limited information available on chlorination of different toxic CB cells, chlorine demand of CB cells, toxins chlorination, and DBP formation potentials in the presence of cellular materials. Moreover, the fate of CB and their associated toxins inside DWTPs (after different conventional and advanced treatment processes) requires further investigation. Australian DW experts are the pioneers in dealing with toxic CB related issues. In order to prepare a management plan for the province of Quebec (Canada), an industrial visit was funded to document Australian experiences dealing with CB related problems.

This dissertation contains three main themes: (1) occurrence and monitoring, (2) treatment and (3) management that will be discussed throughout this document. The general objective of this Ph.D. project is to study the *in vivo* monitoring of CB and their associated toxins in DW sources as well as their potential removal in conventional treatment and oxidation. The specific objectives of this Ph.D. research project are to: (1) Validate the use of online fluorescence probes based on measurements of PC in natural waters; (2) Study the spatial-temporal distribution of CB in Quebec (Canada) water bodies likely of having cyanobacterial proliferation and show the impact of cyanobacterial bloom in the water at the water intake; (3) Monitor the concentrations of CB level and their associated toxins in several DW intakes using a high frequency/event based sampling strategy (e.g.: two times per week, sampling when taste and odour are reported, etc.) using a combination of standard enumeration and online methods; (4) Document the efficacy of chlorine to compromise different cyanobacterial species and oxidize a wide range of cyanotoxins in the pre-treatment stage as well as reduce their toxic potential. Determine and integrate the impact of water quality on models predicting the oxidation of selected cyanotoxins by chlorine;

(5) Study the release of cell-bound organic material and their contribution to the disinfection by-products precursors‟ pool during direct chlorination of intact cells; (6) Analyse the efficiency of cyanobacterial management strategies to prevent cyanobacterial breakthrough in DWTPs implemented by different water authorities in Australia; (7) Detect the potentially toxic CB and their associated toxins in water samples during cyanobacterial bloom formation using ELISA kits and a multi-toxin LC-MS/MS; and (8) Document the removal of CB and their associated toxins (cell-bound and extracellular) through conventional treatment (coagulation, clarification and filtration) and oxidation (chlorine) in pilot and full-scale plants.

The first part of this Ph.D. research project is to study the occurrence of CB and their associated toxins and their monitoring methods. The performance of different available *in vivo* cyanobacterial probes were studied during this project. In order to compare the raw readings of different probes a novel approach was used to present the probe readings in biovolume equivalent of CB. Inorganic turbidity and the presence of algal biomass were found to be two sources of interference involved with probe readings. A correction factor was developed for the probes with simultaneous chlorophyll *a* measurement. The field data demonstrate that the potential underestimation of the cyanobacterial population is a major issue involved with application of *in vivo* probes. Especially, in cases where the underestimation of CB corresponds to actual Alert Levels which are used to trigger monitoring and management actions. Also, the intensive *in vivo* monitoring at the water intake of DWTPs revealed novel information about the impact of CB blooms on these water intakes.

The second section of this dissertation is to study the treatment of CB and their associated toxins using oxidation with chlorine. During the chlorination of *Anabaena circinalis*, with chlorine exposure (CT) value of 7.0 mg.min/L, all cells lost viability causing toxin release. *Anabaena circinalis* cell membrane damage occurred faster than released STXs oxidation. All saxitoxin and more than 95% of other STXs analogues were subsequently oxidized. Kinetic analysis of the oxidation of STXs analogues revealed significant differences in the susceptibility to chlorine, saxitoxin being the easiest to oxidize. In addition, concentrations of trihalomethanes, haloacetic acids, and *N*-Nitrosodimethylamine as chlorination by-products were respectively <50 µg/L and 11 ng/L, even at the highest CT value (50.3 mg.min/L).

During the chlorination of toxic *Microcystis aeruginosa* cells, chlorine exposure (CT) values of 172.4 and 100 mg.min/L were required to obtain 76% cell lysis and oxidation of released cell-bound toxins below guideline value of 1 µg/L MC-LR, respectively. For chlorination experiments in ultrapure water, toxin oxidation rates were similar or faster than cell lysis rates. In most cases, for chlorination experiments in natural water, the cell lysis was faster than toxin oxidation rate. Additionally, concentrations of trihalomethanes (12.87 µg/L) and haloacetic acids (below detection limit) as DBP were below the guideline values in chlorination of 500,000 cell/mL of in *Microcystis aeruginosa* ultrapure water, even with a maximum CT value of 3051.2 mg.min/L. With a chlorine exposure (CT) value of <4.0 mg.min/L >60% cells of toxic strains of *Anabaena circinalis*, *Microcystis aeruginosa*, *Cylindrospermopsis raciborskii* and a non toxic strain of *Aphanizomenon issatsckenka* lost viability, causing toxin release (in cases of toxic cells). Cell-membrane damage occurred faster than oxidation of released toxins. Kinetic analysis of the oxidation of toxins in natural water revealed significant differences in their susceptibility to chlorine, saxitoxins being the easiest to oxidize, followed by cylindrospermopsin and microcystin-LR. Furthermore, concentrations of trihalomethanes and haloacetic acids (<40 µg/L) and N-Nitrosodimethylamine (<10 ng/L) as chlorination by-products were again lower than the guideline values, even at the highest CT value (220 mg.min/L). However, the DBP concentrations in environmental bloom conditions, with very high cell numbers, were over the guideline values.

As a final theme this dissertation examines management challenges of CB and their associated toxins for DW production. The fate of CB cells and their associated toxins has been studied: after the addition of powder activated carbon, post-clarification, within the clarifier sludge, post-filtration and post-chlorination in a full scale DWTP, and in laboratory scale only for chlorination. Accumulation of high cyanobacterial cell numbers (4.7×10^6 cells/mL) and maximum cyanotoxins concentration of 10 mg/L were detected in the clarification process. In one occasion, 2.47 µg/L of microcystins were analyzed in DW which surpassed all guideline values recommended by water authorities across the globe. Direct chlorination of environmental cyanobacterial bloom samples does not reproduce the efficient removal of cells and toxins observed during essays using laboratory cultured cells and dissolved standard toxins.

The six water authorities/industries, visited during the Australia sojourn (March-July 2010), provide water to over 72% of the Australian population as well as four major metropolitan areas.

A questionnaire was prepared and completed in order to document the results of these visits. The major bloom forming CB species in these states are *Microcystis aeruginosa* (producing microcystins), *Anabaena circinalis* (producing saxitoxins) and more recently *Cylindrospermopsis raciborskii* (producing cylindrospermopsin). These water authorities conduct a large scale watershed protection plan by restricting the access to the reservoirs surrounding bushlands. The CB management plans include intensive CB monitoring not only in the water bodies and the close proximity of the water intakes, but also in the tributaries of the reservoirs and major rivers. Application of online CB probes is also included in their monitoring plan. The online monitoring helped them to better understand the CB dynamics in the reservoir and the limitations of different online techniques. These frequent samplings provide them an inclusive historic data. The enhanced monitoring over their water bodies helped them to prevent the flow and accumulation of toxic CB cells in the DWTP. Australian water authorities prepared alternative guidelines derived from existing national and international guidelines that are adapted to their site-specific considerations. They have also implemented site specific guidelines for water reuse and recreational activities.

This dissertation is focused on providing technical information relevant to the needs of the DW industry in managing CB related issues. The laboratory results show that it is possible to achieve conflicting water quality goals (effective toxin oxidation while respecting DBP guidelines) using chlorination conditions that would be experienced in a DWTP. However, operators of DWTPs have to be prudent with the interpretation of laboratory findings in operational conditions. Water authorities need to adapt technical tools and thresholds to site-specific issues to develop practical management and intervention tools suited to local problems. Documentation of the particular local problems and site specific needs are a key factor in this procedure.

CONDENSÉ EN FRANÇAIS

Les cyanobactéries (CBs) se retrouvent naturellement dans l'écosystème aquatique. La prolifération excessive des CBs toxiques constitue l'une des conséquences possibles de l'eutrophisation des lacs, des rivières et des réservoirs d'eau. Lorsque des conditions environnementales particulières (tel que la lumière, la température et la stabilité de la colonne de l'eau) conviennent à leur croissance, ses CBs peuvent proliférer massivement et former des fleurs toxiques en surface de l'eau. Ce phénomène de prolifération, appelée "Bloom", est souvent accompagné par une libération de diverses substances toxiques appelées cyanotoxines, qui peuvent avoir différents impacts aussi bien écologiques que sanitaires. En effet, beaucoup d'espèces de CBs peuvent synthétiser un important éventail de cyanotoxines nuisibles.

Prolifération cyanobacteriènne, potentiellement toxique, est généralement favorisée par un réchauffement climatique. L'augmentation de la température et des ruissellements associés aux changements climatiques exacerbe l'impact de ces apports. Elle favorise une spéciation algale en faveur des espèces nuisibles qui produisent des toxines et des composés générateurs d'odeurs et de goût désagréables (MIB, geosmine, etc.) au niveau des eaux des sources d'eau potable. L'Organisation mondiale de la santé (OMS) a émis une directive de norme provisoire de 1 µg/L en équivalent de microcystines-LR, l'une des variantes de toxines cyanobactérienne la plus fréquente. Il s'agit de cyanotoxines auxquelles une attention particulière a été donnée, non seulement en raison de leur grande capacité de causer des intoxications aigues, mais également en raison de leur potentiel cancérigène par l'exposition chronique de la population à de faibles concentrations de microcystines dans l'eau potable.

Plusieurs cas d'intoxications touchant à la fois les animaux d'élevage et l'homme ont été rapportés dans plusieurs pays (Australie, Brésil, États Unis, Canada, Royaume-Uni, Afrique du sud, etc.). Chez l'homme, des cas de dermatites, de conjonctivites, de pneumonies ainsi que des troubles hépatiques et intestinaux ont été attribués à des baignades ou à la consommation d'eaux de boisson distribuées à partir de réservoirs contenant des CBs toxiques. Suite à la multiplication de ces problèmes dans le monde, l'étude des pullulations toxiques à CBs a été entamée depuis les 25 dernières années.

Cette thèse de doctorat comporte trois thèmes principaux qui seront développés dans ce document : (1) présence et surveillance (2) traitement et (3) gestion. L'objectif général de ce

doctorat est d'étudier la surveillance *in vivo* de CB et de leurs toxines dans les sources d'eau potable ainsi que leur élimination potentielle par traitement conventionnel et l'oxydation. Les objectifs spécifiques de ce projet de recherche sont les suivants : (1) valider l'utilisation de sondes de fluorescence *in vivo* basée sur des mesures de PC dans les eaux naturelles (2) étudier la distribution spatio-temporelle de CBs dans les masses d'eau susceptible d'avoir une prolifération de CBs au Québec, et de montrer l'impact de cette prolifération dans l'eau à la prise d'eau; (3) surveiller les concentrations de CBs et leurs toxines dans plusieurs prises d'eau potable en utilisant des stratégies d'échantillonnage à haute fréquence et par événement (par exemple : échantillonner deux fois par semaine, l'échantillonnage lorsque le goût et l'odeur sont rapportés, etc.) avec une combinaison de dénombrement usuel et des méthodes en ligne; (4) documenter l'efficacité du chlore à compromettre les différentes espèces de CBs, à oxyder une large gamme de cyanotoxines dans l'étape de prétraitement, et à réduire leurs potentiels toxiques. Déterminer et intégrer l'impact sur la qualité d'eau dans les modèles de prévision d'oxydation de cyanotoxines par le chlore; (5) étudier le relargage des matières organiques liées à la cellule, et aussi leur contribution aux précurseurs des sous-produits de désinfection lors de la chloration directe de cellules intactes; (6) analyser l'efficacité des stratégies de gestion de CBs appliqué par les différentes autorités d'eau en Australie pour empêcher la percée de CBs dans les usines de traitement d'eau potable; (7) pendant la formation de prolifération de CBs, détecter leurs potentielle toxiques et leurs toxines dans des échantillons d'eau en utilisant des kits ELISA et une méthode LC-MS/MS multitoxine, et (8) dans une usine de traitement à pilote et grande échelle, documenter l'enlèvement de CB et leurs toxines (intra - et extracellulaire) grâce à un traitement conventionnelle (coagulation, clarification et filtration) et d'oxydation (chlore).

Le premier thème sur l'occurrence et surveillance des épisodes de prolifération de CBs est présenté au chapitre 4. L'estimation rapide des concentrations de CBs sur le terrain est une activité nécessaire à la gestion efficace des risques associés à la présence de cyanotoxines dans certaines activités de récréation et dans la consommation d'eau potable. Les méthodes actuelles de détection des CBs sont fastidieuses, coûteuses et requièrent des délais d'analyse importants. Elles sont trop lourdes pour localiser et suivre l'évolution des efflorescences *in situ* et ne sont pas à la portée des municipalités. La mesure de la phycocyanine (PC) *in vivo* par des sondes immergeables constitue une approche d'intérêt pour l'estimation et l'évolution de la biomasse des CBs. Ce type de mesure présente de grands avantages dont: la spécificité, la simplicité

relative d'opération, la rapidité des prises de mesure, la nature portable qui sont bien adaptées à l'établissement de profil à plusieurs points dans la source, la facilité d'utilisation en continu et le coût relativement modeste. Toutefois, leur utilisation dans un cadre de gestion soulève certaines questions quant à leur précision et leur spécificité dans des conditions environnementales. Ces questions deviennent particulièrement pertinentes dans l'optique de l'application de cette technologie pour déterminer l'atteinte de niveaux d'alerte et d'intervention. Dans le cas de seuils définis par les nombres ou la biomasse de CBs, l'utilisation de la PC *in situ* requiert l'utilisation de facteurs de conversion pour exprimer la fluorescence relative en concentration de pigment, en nombre ou en biomasse de CBs. Or ces corrélations sont complexes et peu documentées. De plus, peu d'information existe sur les procédures de calibration et d'interprétation des données générées par ce type de sonde, ou sur l'intérêt et l'utilité de mesurer plusieurs autres paramètres simultanément.

Diverses sondes de PC *in vivo* sont utilisées pour détecter les CBs potentiellement toxiques dans des eaux de surface. L`industrie d`eau potable à travers le monde utilise ces sondes pour la surveillance et la gestion. Par conséquent, un certain nombre de problèmes concernant la calibration, la précision, les biais, l'interprétation des données, la nécessité des mesures simultanées de Chl*a in vivo* et turbidité doivent être abordés pour une utilisation efficiente de ces sondes. C'est ainsi qu'en 2008, un projet a été initié par l'École Polytechnique de Montréal (ÉPM) afin de mener des études sur ces sondes de PC dans le but d'élaborer une stratégie pour leur utilisation dans le cadre de mesures faites sur le terrain. La première phase du projet réalisé en collaboration avec l`« Australian Water Quality Center » a permis de déterminer les principales sources d'interférences et d'apporter des facteurs de correction. La deuxième phase du projet achevé à l'ÉPM en 2009-2010 avait pour objectifs: (1) d'étudier la précision des mesures de CB avec deux sondes de PC disponibles sur le marché (2) de valider une nouvelle stratégie combinant des sondes de PC et Chla *in vivo* avec des mesures des microcystines (MC) pour la surveillance des CBs toxiques dans 12 sources d'approvisionnement d'eau.

Les mesures au laboratoire pour valider les sondes ont été effectuées sur les suspensions mono et mixtes de CB (*Microcystis aeruginosa* et *Anabaena circinalis*) et de Chlorophyta (*Scenedesmus* et *Pseudokirchneriella subcapitata*) en utilisant les sondes de PC YSI6131 et Chl*a* YSI6025 d'Yellow Springs Instrument (YSI, Yellow Springs, Ohio, USA), de PC TriOS-MicroFlu-bleu (Oldenburg, Germany) et biological·biophysical·engineering (bbe) Moldaenke (Kronshagen,

Germany). Les Kaolins et Bentonite ont été utilisés pour simuler une gamme de turbidités inorganiques (1-70NTU) pour la validation de la sonde. Au cours de l'été et durant l'automne 2008-2010, des suivis concernant les CB ont été effectués dans plusieurs lacs/rivières/réservoirs, y compris le lac Champlain, au Québec. Certaines mesures des sondes ont été faites en parallèle avec une énumération microscopique, l'estimation du biovolume et des analyses de MC (Kit ELISA). Les données brutes des sondes PC ont été comparées au nombre de cellules/mL et les estimations du biovolume.

Les lectures des trois sondes suivent une tendance linéaire, même en présence de concentrations élevées de CB. Les concentrations de Chlorophyta et turbidités minérales ont induit des biais sur les lectures des sondes PC *in vivo*. Les résultats montrent des corrélations significatives entre données brutes des sondes PC *in vivo* et biovolume des CB potentiellement toxiques. Aussi une bonne corrélation a été observée entre les concentrations totales de MC et le biovolume de CB. En outre, l'utilisation de la corrélation entre les données brutes des sondes PC *in vivo* et les valeurs de biovolume de CB interprétées avec les données de Chl*a* s'avère un indicateur utile pour surveiller des CB.

Ce projet présente les informations requises pour les usines de traitement d'eau potable et les autorités chargées de la protection des sources qui veulent appliquer ces sondes pour la surveillance *in vivo* des CBs. Les résultats démontrent également que la combinaison des sondes de PC et de Chla appliquées à la surveillance de l'eau brute à l'entrée des usines de traitement d'eau potable est préférable pour définir la réponse adéquate pour le traitement des proliférations.

Le deuxième thème, traitement avec oxydation, est présenté aux chapitres 5, 6 et 7. De plus, le troisième thème sur la gestion des CBs et leurs toxines associées sont présentés au chapitre 8 et l'annexe F. La prolifération des CBs d'eau douce (CB) au Québec sont généralement dominés par les espèces productrices de microcystines (MC) et anatoxines (e.g. *Microcystis aeruginosa*). Toutefois, les CB fleurs dans l'Australie du Sud sont généralement dominées par *Anabaena circinalis*, qui produit des « Paralytic Shellfish Poisons » ou Saxitoxines (STXs: Saxitoxine, GTX and C toxines). STXs ont été trouvés dans certaines sources d'eau douce en Amérique du Nord également. En Amérique du Nord, plusieurs sources sont non-filtrés et pourrait être de plus en plus l'objet d'efflorescences de CBs en raison du changement climatique. Par conséquent, il

est nécessaire de comprendre les limites de l'oxydation des cellules de CBs et de leurs toxines. L'impact de la chloration sur les cellules d'*A. circinalis* et *M. aeruginosa* a été étudiée pour documenter la lyse cellulaire, le relargage et l'oxydation de toxines.

Les premiers essais d'oxydation de toxine ont été effectués en utilisant un extrait naturel concentré de PSP dosé en eau Milli-Q et eau de rivière Murray (Australie du Sud). Les eaux dosées par les toxines ont été chlorées (3 mg/L) pour un maximum de temps de contact d'une heure à pH ambiant et contrôlé (pH 8) durant lequel des échantillons ont été prélevés pour des analyses de PSP. La première phase des essais de l'oxydation des cellules ont été effectués en utilisant une souche d'*A. circinalis* productrice de PSP ensemencée en Murray River, à une densité de 50000 cellules/mL. Ensuite, cette eau (tamponnée à un pH de 8) a été chlorée à 2-3 mg/L (résultant des CT de 0-25 mg.min/L). Pour la deuxième phase, la même densité d'une souche de M. aeruginosa productrice de MC a été ensemencée dans la rivière des Milles Iles. Les échantillonnes d'eau résultante (tamponnée à pH 7 et 8,5) ont été chlorées à 2, 5 et 10 mg/L. Pour les deux phases, les échantillons d'eau ont été trempé/quenché avec du thiosulfate de sodium après des intervalles de temps prédéfinis et ont été analysés pour les paramètres suivant : nombre total des cellules, tests d'intégrité cellulaire (en utilisant Fluorescein diacetate/Propidium iodide (FDA-PI)), géosmine et des teneurs en toxines.

Les deuxièmes séries d'essais d'oxydation cellulaire au laboratoire ont été réalisées en utilisant des souches de *M. aeruginosa*, *A. circinalis*, *Cylindrospermopsis* (trois espèces toxiques) et *Aphanizomenon* (non-toxique) qui ont été prélevées dans l'eau brute de la rivière des Mille-Îles (Montréal, Canada) et celle de Prospect (Sydney, Australie) avec les densités de 50000-200000 cellules/mL. Les échantillons d'eau (tamponnée à pH 7 et 8,5) ont ensuite été chlorées à 2-5-10 mg/L (résultant des CT de 0-380 mg.min / L). Après un temps prédéfini de contact, le chlore résiduel était réduit à l'aide de thiosulfate de sodium. Le test de viabilité cellulaire, l'énumération microscopique des cellules totales et l'analyse des toxines ont tous été effectués sur des échantillons. Des échantillons d'eau de deux cas de fleurs d'eau de CB respectivement dans le lac Champlain (Canada) et la rivière Torrens (Adélaïde, Australie) ont également été chlorés à 2 et 5 mg/L avec pH ambiant.

L'exposition des cellules d'*A. circinalis* à de faibles doses de chlore a diminué immédiatement le nombre de cellules intactes (FDA+), mais n'a pas causé de lyse significative (<25%). Les

cellules viables, mais endommagées (FDA-/PI-) apparues immédiatement après la chloration, même à faible CT (<5 mg.min/L), ceci constituent une étape intermédiaire. La perte de la viabilité après chloration, telle que mesurée par le PI+, est soumise à l'interprétation de l'intensité de fluorochrome. Avec les CTs plus élevées (> 10 mg.min/L) presque toutes les cellules ont perdu la viabilité (PI+), mais n'ont pas lyser. Les dommages de paroi cellulaire ont été associés à des relargages rapides (<3 min) de géosmine. Les doses modestes de chlore (2-3 mg/L) ont causé peu ou pas de lyse cellulaire, mais ont été suffisantes pour provoquer le relargage de toxines et de géosmine. Des STXs relargés, tout le STX et plus de 95% de C toxines et GTX ont ensuite été oxydé par la chloration dans ces conditions de chloration (CT < 25 mg.min/L). L'exposition de M. aeruginosa à trois dosages de chlore dans les différentes conditions de pH a causé le relargage rapide des toxines dans les deux premières minutes de temps de contact. Ainsi, après trente minutes de contact la diminution de la MC à un niveau sous le seuil de l'OMS, 1 µg/L, a été observé.

Dans les conditions environnementales réelles, la concentration des CBs dans la prise d'eau d'une usine de traitement d`eau potable située sur le côté canadien du lac Champlain a été suivie durant l'été 2008-09. Les tests ont débuté dès que la concentration des CBs dans l'eau brute a dépassé le seuil d'alerte fixé au Québec (20000 cellules/mL). La densité cellulaire et le biovolume des CBs ainsi que la concentration de MC ont été suivies: (1) au point d'ajout de produits chimiques (2) après décantation; (3) dans le lit de boues avec le CAP; (4) après filtration, et (5) postchloration.

Lorsque les densités cellulaires élevées de CBs ont été enregistrées à la prise d'eau (> 100000 cellules/mL) le suivi des CBs dans le chaine de traitement a révélé d'importantes accumulations de CBs (> $5{,}4\times10^6$ cells/mL) dans le lit de boue (les CB ont été inclus dans les grandes flocs). Les concentrations de MC-LR eq. dans le lit de boues ont dépassées 1µg/L (recommandation de l'OMS en eau potable) de près de 40 fois. Moins de 2500 cellules/mL étaient restées après la filtration et dans l'eau chlorée. La concentration de MC-LR eq. dans l'eau chlorée a été inférieur à <1µg/L. Ces résultats sont en accord avec les observations des autres publications sur des percées des cyanotoxines dans les usines de traitement d`eau potable.

Des échantillons d'eaux prélevés lors de fortes prolifération de CBs au Canada (été 2009 - hiver 2010) ont été exposés à des CTs plus faibles que 25mg.min/L. Ces dernières n'ont pas causé de dommages cellulaires, il n'y a pas eu de libération ni d'oxydation de toxines. Les CTs de 25 à 90

mg.min/L ont été suffisant pour endommager les membranes des cellules de M. aeruginosa, induisant le relargage des toxines et l'oxydation subséquente de toxines (> 99%). Les tests de viabilité cellulaire ont confirmé qu'avec les CTs > 25 mg.min/L toutes les cellules ont été endommagées ou métaboliquement inactifs. Toutefois, ils n'ont pas été complètement dégradés. Ces résultats confirment que les cellules observées dans l'eau chlorée de l`usine ont été endommagées ou métaboliquement inactifs et ne contiennent pas de toxines. Pourtant, la chloration directe d'échantillons des fleurs d`eau environnementales en utilisant les mêmes valeurs de CT n'a causé qu'une dégradation de 20% de biovolume totaux de CB et une oxydation de 38% des toxines totaux. Des expériences similaires en Australie (mars-juin 2010) ont confirmé les résultats que celles au Canada.

Les résultats montrent que de faibles doses de chlore ont abouti au relargage rapide et l'oxydation subséquente de toxines de deux souches de cyanobactéries toxiques. En outre, ces résultats ont montré que le traitement efficace des toxines peut se produire sans compléter la lyse cellulaire. Ainsi, il est essentiel d'être prudent avec l'interprétation des résultats de laboratoire et leur application dans des cas réels des usines de traitement d`eau potable, même si la chloration est une barrière prometteuse.

De nouvelles directives et normes de toxines de cyanobactéries ont récemment été promulguées et une norme de 1,5 µg/L a été proposée dans la mise à jour des règlements sur la qualité d'eau potable pour les microcystines (ministère du Développement durable de l'Environnement et des Parcs (MDDEP) 2010). La gestion des cyanotoxines fait aussi l'objet de recommandations dans le guide de conception des installations de production d'eau potable au Québec. Les stations d'eau potable doivent évaluer la présence de proliférations de cyanobactéries (CB) toxiques dans leur source, ajuster leur traitement et évaluer le risque de percée de cyanotoxines dans l'eau traitée.

Les suivis récents des CB dans plusieurs usines de traitement d'eau potable au Québec ont mis en évidence la présence d'écumes de CB toxiques et leur accumulation dans certains procédés. Ces résultats montrent que les CBs peuvent s'accumuler et peut-être même se développer dans les clarificateurs et les filtres. L'accumulation de cellules des CB toxiques dans les usines de traitement d'eau potable pourrait conduire à la percée de cyanotoxines dans les eaux traitées. L'ampleur de ces accumulations et le potentiel de relargage de toxines associés à ces

accumulations méritent donc d'être quantifiés. Les risques associés à cette accumulation pourront ensuite être estimés ainsi que les mesures correctives identifiées.

L'usine de Bedford puise son eau brute dans la baie de Missisquoi du Lac Champlain qui est sujet à des proliférations de cyanobactéries. La concentration de cyanobactéries à l'eau brute varie de façon marquée et n'est pas généralement associée à une concentration élevée de toxines algales. Toutefois, des mesures plus fréquentes en 2008 et 2009 ont révélé que des concentrations de toxines élevées peuvent être présentes à l'eau brute.

En 2010, des concentrations de 119 µg/L à l'eau brute, de 171 à 10,331 µg/L dans des écumes et de 24 µg/L dans le lit de boues ont été mesurées. Les événements semblables ont été observés en 2008 et 2009 dans l'usine de Bedford. L'accumulation de cellules de cyanobactéries dans les procédés de l'usine peut donc amplifier le risque présent à l'eau brute. Malgré le fait que le MDDEP ait suivi les niveaux de cyanobactéries et de toxines régulièrement à l'usine de Bedford depuis 2002, des concentrations élevées de toxines n'avaient pas été mesurées. Il est possible que les calendriers d'échantillonnages ne coïncident pas forcément avec les périodes de pointe de toxines.

Un des éléments à retenir de ces observations est la concentration faible de 1,72 µg/L de MC-LR à l'eau traitée. Cette concentration dépasse les niveaux recommandés par Santé Canada et l'Institut National Santé publique du Québec (INSPQ) de 1,5 µg/L. Or la chloration est particulièrement efficace pour éliminer la MC- LR. Par contre, la chloration n'est pas efficace pour éliminer d'autres cyanotoxines comme l'anatoxine a. Ces observations montrent l'importance de :

(1) cibler les périodes d'efflorescence maximale pour effectuer la caractérisation du risque pour les cyanobactéries, et

(2) de vérifier si ce risque peut être amplifié dans les stations de traitement des eaux potables.

Les observations à Bedford mettent en évidence la vulnérabilité potentielle d'autres prises d'eau prise et l'intérêt d'effectuer un suivi plus intensif dans des sites considérés à risque. Très peu d'informations sont disponibles sur la durée et la fréquence des évènements de pointes de cyanobactéries et de cyanotoxines à l'eau brute, leur intensité et les répercussions subséquentes

au long de la filière de traitement. En plus, les informations sur la capacité des procédés de traitement par décantation (temps de rétention hydraulique et temps de rétention des solides) sont contradictoires.

Les succès des Australiens en matière de gestion des problématiques des CB sont dus :

(1) aux programmes de suivie *in vivo* intensif des CB avec les prises des échantillons pour valider les méthodes *in vivo*;

(2) à l'application des guides de gestion pratique adaptée aux besoins particuliers des sites.

Les conclusions de ce rapport et les propositions pertinentes pour la situation au Québec est résumée en deux parties:

(1) une première conclusion souligne la nécessité de connaitre l'ampleur des problématiques des CB dans les régions concernées par l'analyse de données historiques et l'acquisition de nouvelles données. Pour acquérir ces nouvelles données, l'utilisation des méthodes de mesure intensive *in vivo* en parallèle avec les méthodes conventionnelles est essentielle. Les méthodes *in vivo* ont le très grand avantage de fournir des données *in situ* à haute fréquence (chaque 30 minutes).

En plus, un programme de surveillance globale (de la source au robinet des consommateurs) pour assurer la santé publique est fortement recommandé. Basés sur les informations de suivi des CB dans les plans d`eau australiens, nous proposons des études pour le suivi systématique (en utilisant la technologie des sondes *in vivo*) de la présence des CB dans les usines de traitement d`eau potable au Québec (e.g. à la prise d`eau, dans les décanteurs, sur les filtres). Ces études viseront à documenter cette problématique particulière et à développer des outils de gestion et d'intervention spécifiques aux problèmes des usines québécoises.

(2) Une deuxième conclusion établit la nécessité de prendre connaissance des besoins des individus et organisations touchées par la problématique des CB et de mesurer leur taux de satisfaction face aux plans de gestion et les seuils actuels. Basé sur les rencontres avec les experts australiens, nous proposons un projet pour enquêter sur l'état de la connaissance de l'existence des guides d'interventions actuels au Québec et si celui-ci

répond à leurs besoins particuliers. L'adaptation des solutions scientifiques et techniques et des seuils avec les besoins locaux pour un guide pratique est un bon investissement pour l'avenir de la santé publique.

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ANTX	Anatoxin
ANA	*Anabaena* sp.
APHAC	*Aphanothece* sp.
APHAZ	*Aphanizomenon* sp.
AWQC	Australian Water Quality Centre
C1	C-toxin1
C2	C-toxin2
CA	California
CB	Cyanobacteria
CCL	Contaminant Candidate List
Chl	Chlorophyll
Chl*a*	Chlorophyll *a*
CYN or CYL	Cylindrospermopsin
DAF	Dissolved Air Flotation
DBP	Disinfection by-Product
DOC	Dissolved Organic Carbon
dmMC	3-desmethylmicrocystins
DW	Drinking Water
DWTP	Drinking Water Treatment Plant
EPM	École Polytechnique de Montréal
FDA	Fluorescein Diacetate
GAC	Granular Activated Carbon
GTX	Gonyautoxin

GV	Guideline Value
HAA	Haloacetic Acids
HILIC	Hydrophilic Interaction Liquid Chromatography
HPLC	High Performance Liquid Chromatography
INSPQ	Institut National de Santé Publique du Québec
IVF	*In vivo* fluorescence
LC	Liquid Chromatography
LD_{50}	Lethal Dose 50%
LED	Light-Emitting Diode
LPS	Lipopolysaccharides
MA	Massachusetts
MAC	Maximum Allowable Concentration
MB	Missisquoi Bay
MCs	Microcystins
MC	Microcystin
ME	Maine
MIB	Methyl Isoborneol
MIC	*Microcystis* sp.
MIR	Mille-Îles River
MR	Murray River
MS	Mass Spectrometry
NDMA	N-Nitrosodimethylamine
NOAEL	No Observed Adverse Effect Level
NY	New York

OR	Oregon
PAC	Powdered Activated Carbon
PC	Phycocyanin
PDA	Photodiode Array
PE	Phycoerythrin
PI	Propidium Iodide
P_{intake}	Intake Percentage by Human
PPIA	Protein Phosphatase Inhibition Assay
PSP	Paralytic Shellfish Poison
PST	Paralytic Shellfish Toxin
STXs	Saxitoxins
STX	Saxitoxin
T&O	Taste and Odor
TDI	Tolerable Daily Intake
THM	Trihalomethanes
TR	Torrens River
TTHM	Total THM
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation

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CHAPTER 1 INTRODUCTION

Cyanobacteria (CB), also known as blue-green algae, are prokaryotic photosynthetic micro-organisms present in most ecosystems. They are asexual phytoplanktons with gram-negative cell walls (AFSSA-AFSSET, 2006); Hudnell, 2006). Their pigmentation can vary from blue-green to red. Unlike eukaryotic algae, CB possess only one kind of chlorophyll, that is, chlorophyll a (Chl*a*), as a photochemically active pigment. CB possess a unique set of accessory pigments of the phycobiliprotein family, constituting the organism‟s main light absorption complex. The primary phycobilin pigments are phycocyanin (PC) and phycoerythrin (PE), and both happen to have strong fluorescent signatures (λ_{max} 620 nm with far red to red fluorescence for PC and λ_{max} = 565 nm with orange to reddish fluorescence for PE) that do not interfere significantly with the fluorescence of the chlorophylls (Appendix A). CB with the PC phycobilin pigment can be found in both fresh and brackish water environments, while CB with the PE phycobilin pigment is usually found only in brackish or marine environments (Carr & Whitton, 1982; Fogg et al., 1973). Although the pigment content may vary according to the physiological state of the organisms (e.g. it increases if light availability is low), the global evolution of the pigment is the same as that in the biomass (Cagnard et al., 2006; Chorus & Bartram, 1999).

CB have long been recognised as notable for their nitrogen fixing capacity (the ability to convert atmospheric N_2 to NH_3), which contributes globally to soil and water fertility, and also for the fact that they are probably one of the primary organisms responsible for providing an oxygen-rich atmosphere on Earth (Chorus & Bartram, 1999; Svrcek & Smith, 2004) . It is estimated that they have been present in our planet‟s life cycle for over 3.5 billion years (Hudnell, 2006). In the last 20 years, most of the literature covering CB has labelled them as a potent producer of a variety of toxins responsible for intermittent but repeated widespread poisoning of wild and domestic animals, aquacultured fish and, more recently, humans (Carmichael et al., 2001). Many potentially toxic species of CB and their associated toxins have been detected (Appendix B).

The most undesirable effects of CB bloom events (Appendix A) for human consumption and activities is summarized as follows (AFSSA-AFSSET, 2006; Newcombe et al., 2010):

- Skin or mucous membrane problems as a result of bathing in waters affected by blooms.

- Complication of the DW treatment process, in particular mechanical stages such as filters or membrane clogging.
- Sudden changes in raw water pH, disruption of flocculation reactions, increasing the consumption of coagulant and generation of disinfection by-products (DBP) due to organic materials involved with bloom matrix.
- Disruption of dialysis equipment by accelerated clogging if the water treatment is inadequate.
- Taste and odour (T&O) production.
- Toxins synthesizing.

CB grow and produce their toxins in surface waters throughout the world, particularly under eutrophic conditions, but also in the metalimnion of deep mesotrophic reservoirs. Many of these surface waters are used for DW production. While it is not known for certain why CB produce toxins, it has been surmised that they are evolutionary carry-overs and may function as protective secretions, as researchers have shown some cyanotoxins to be potent inhibitors of aquatic invertebrate grazers (Carmichael et al., 1997; Chorus & Bartram, 1999; Gijsbertsen-Abrahamse et al., 2006).

In bloom events, more CB biomass indicates a higher probability of toxin presence. The biosynthesis of cyanotoxins is an energy-intensive process, and the toxic properties of these molecules may have nothing to do with their functions (Wilhelm et al., 2007). Cyanotoxins are largely retained within the cyanobacterial producer-cell during its growth phase with less than 10 to 30% as extra-cellular. A sudden increase in the concentration of dissolved toxins in the water column can occur after the collapse of a bloom and consequent cell lysis (Jones & Orr, 1994; Hyenstrand et al., 2003).

The occurrence of toxic CB bloom events in water sources is a growing concern worldwide and toxic CB are increasingly found in high densities at the water intakes of DWTPs. Particularly, more northern industrial countries are now facing CB bloom related issues. A factor considered to explain this increase in occurrence of CB bloom events is global climate change. According to the available information, climate change could increase the frequency and intensity of CB blooms due to increase in water temperature, growth season, runoff and nutrients availability. CB

toxins have recently been included in guidelines and regulations and are now listed on the United States Environmental Protection Agency (USEPA) Contaminant Candidate List (CCL). Utilities must evaluate the presence of toxic cyanobacterial blooms in their source water in order to assess the risk of cyanotoxins passage in treated water.

CB are generally monitored by low frequency sampling programs. Transient localized events of potential significance for treatment may not be detected using such a sampling approach. On the other hand, spatial accumulation of CB varies significantly in a source, and the presence of CB in one area of a source may not necessarily affect the concentration of CB in the water intake. A systematic monitoring plan is needed to study the short term variations of CB in source waters. Various online *in vivo* PC probes are used to detect potentially toxic CB in source waters. Industry across the world is readily adopting these probes for monitoring and management. However, water quality may interfere with probe measurements in natural environments. Therefore, the issues of calibration, accuracy, biases, data interpretation, the need for simultaneous of *in vivo* Chl*a* and turbidity must be addressed.

Once toxic CB and/or cyanotoxins are detected, proper treatment methods must be applied for their removal depending on water quality for DW production. In the case of cyanotoxins, it is important to consider dissolved molecules and cell-bound toxins, which can be released at the time of cellular lysis. Of the total quantity of toxins measured in an unfiltered sample, approximately 70% are contained within intact cyanobacterial cells, and the remaining 30% are dissolved toxins in the water (Donati et al., 1994). In most guidelines, however, it is not clear if the cell-bound and extra-cellular toxins should be analysed separately or additively. It is necessary to understand the limitations of the treatment options in dealing with dissolved and intracellular toxins.

It has been shown that, despite the presence of phytoplankton cells and heterotrophic bacteria, cyanobacterial hepatotoxins, lipopolysaccharide endotoxins and large cyanobacterial cells were effectively removed at a DWTP using coagulation, clarification, sand filtration, ozonation, slow sand filtration and chlorination (Rapala et al., 2006). Most literature favours the methods that trap the biomass without liberating the toxins before the oxidation stage. Conventional treatment processes, including coagulation, flocculation, sedimentation and filtration, will remove up to 90% of the total STXs, MCs and CYN, if they are contained within healthy cyanobacterial cells

(less for cylindrospermopsin). However, dissolved toxins must be removed using additional treatment steps such as oxidation by ozone, chlorine and potassium permanganate (Daly et al., 2007; Lin et al., 2009; The Cooperative Research Centre for Water Quality and Treatment (Australia), 2007). It is necessary to understand if the presence of toxic CB cells in the DWTPs might lead to (1) passage of cyanotoxins in treated water and (2) significant accumulation of CB cells in different treatment processes. In case of a potential accumulation, the risk associated with this phenomenon needs to be quantified and remedial measures identified.

In North America, unfiltered DW is produced from several protected and semi-protected sources and disinfection is applied directly to raw water. In the United States of America (USA), unfiltered sources serve approximately 11,000,000 customers and include cities such as New York City (NY), Boston (MA), Portland (ME), Portland (OR), San Francisco (CA) (S. Regli, *pers. comm.*). In Quebec (Canada) alone, there are 130 small and very small utilities which produce unfiltered DW (Ministère du Développement Durable de l'Environnement et des Parcs (MDDEP), 2008). Unfiltered plants typically apply single or dual disinfection, usually chlorination, ozonation and/or UV oxidation, directly to raw water that may contain CB cells. Pre-oxidation ahead of filtration is commonly used in water treatment for many purposes, including manganese and iron removal, optimized particle removal (including the reduction of algal cells), and filter cycle optimization (Chen & Yeh, 2005; Mouchet & Bonnelye, 1998; Petrusevski et al., 1995; von Sperling et al., 2008). Furthermore, in 97% of DWTPs in Quebec pre/post-chlorination is used for DW production (Barbeau et al., 2009). It is anticipated that climate change will increase the frequency and intensity of cyanobacterial blooms thus impacting DW intakes (Elliott et al., 2006; Jöhnk et al., 2008). DWTP producing unfiltered DW and plants relying on pre-oxidation will be especially vulnerable to more frequent bloom events (Barbeau et al., 2009). Therefore, there is a need to understand the limitations of the oxidation of CB cells and their toxins.

The health relevance of CB and their associated toxins, the treatment challenges to remove these compounds, and the observed trends of growing predominance of CB in surface water all require a comprehensive study of monitoring, treatment and management options. Furthermore, a good understanding of (a) the recorded historic occurrence of CB and their associated toxins in water sources across the globe and particularly in Nordic regions and (b) their impact on the intake of

DWTPs and DW production are essential for a comprehensive management plan. Consequently, the scientific material of this Ph.D. dissertation is presented within three main themes:

1. Occurrence and monitoring of CB and their associated toxins.
2. Treatment options for removal of CB and their associated toxins focusing on oxidation with chlorine.
3. Management of CB and their associated toxins related issues for DW production.

This document presents a critical review of the literature, the hypothesis and objectives of the Ph.D. research, the research approach, five scientific articles, a general discussion and conclusion. The core of this paper-based dissertation is to present the scientific publications of this research. During this Ph.D. two papers have been published in the Journal of Environmental Science and Technology, and the Journal of Water Research. Also, two manuscripts have been submitted for publication to the Journal of American Water Works Association and the Journal of Water Research. Furthermore, a fifth manuscript is presented as supplementary information on the oxidation of CBs and their associated toxins.

CHAPTER 2 CRITICAL REVIEW OF THE LITERATURES

The main objective of the critical review of published research is to figure out the missing information about occurrence and monitoring (section 2.1), treatment (section 2.2) and management (section 2.3) of potentially toxic CB cells and cyanotoxins in drinking water sources.

2.1 Occurrence and monitoring of CB and their associated toxins

It is necessary to study the records of the historic occurrence of CB and their associated toxins before conducting a new monitoring programme. Therefore, this section starts with a review of the recorded occurrences and then continues with the review of monitoring methods.

2.1.1 Occurrence of CB and their associated toxins

Cyanotoxins production, release of T&O and DW treatment process perturbation are the main challenges that the water industry is facing due to CB occurrence (Merel et al. 2010). T&O are specific indicators of cyanobacterial proliferation in raw water. *Anabaena* sp., *Aphanizomenon* sp., *Microcystis* sp., *Oscillatoria* sp. and *Planktothrix* sp. were linked with two odour compounds, geosmin (trans-1,10-dimethyl-trans-9-decalol) and 2-Methylisoborneol (MIB), in waters effected by cyanobacterial bloom (Baudin et al., 2007; Li, Wan et al., 2007; Lin et al., 2009). These two earthy-musty smelling metabolites are slightly polar, relatively low molecular weight aliphatic tertiary alcohols with similar structures and solubility (Newcombe et al., 2010). Meanwhile, geosmin and MIB are observed in bloom events containing MCs toxicity, but the current knowledge provides no direct correlation between cyanotoxins and T&O (Carmichael et al., 2001; Li et al., 2007).

CB are capable of producing several classes of toxins. Cyanotoxins are classified in Table 2.1 according to their toxic effect and production. While there are several potential exposure routes to cyanotoxins, the most common human exposure pathway is the ingestion of water, both as a daily DW requirement and accidental recreational intake. Chemical structures of different cyanotoxins are presented in Appendix C.

Most of the knowledge about the toxicity and dose-response relationships of the various cyanotoxins comes from laboratory tests on mice and rats. Table 2.2 summarizes the lethal

dosages for each of the major cyanotoxins. The acute toxicity is the bases of toxicity tests. With regard to the ingestion of water contaminated by CB, the observed effects are stomach cramps, vomiting, diarrhoea, fever, headache, hepatoenteritis, atypical pneumonia, pains in muscles and joints, and weakness. Furthermore, these effects were observed as a result of accidental ingestions during episodes of swimming (Health Canada, 2002). The primary types of toxicities when exposure to cyanotoxins occurs include acute hepatotoxicosis, peracute neurotoxicosis, gastrointestinal disturbances, and respiratory and allergic reactions (Svrcek & Smith, 2004).

Acute hepatotoxicosis, caused by hepatotoxins, is the most commonly encountered toxicity involving CB. Infrequent, but repeated, cases of wild and domestic animal poisonings, especially among cattle, sheep, horses, pigs, and ducks still comprise the main problem involving hepatotoxic cyanotoxins. The symptoms of hepatotoxic poisoning include weakness, anorexia, pallor, cold extremities, laboured breathing, vomiting and diarrhoea. Death occurs within a few hours to a few days after initial exposure and may be preceded by coma, muscle tremors, and forced expiration of air (Svrcek & Smith, 2004).

The acute mode of hepatotoxic action is to cause cell–cell separations in the liver cells (hepatocytes), allowing for accumulation of blood in the liver and eventual death of the animal by haemorrhagic (internal bleeding) shock or liver failure. The cyclic peptides have been shown to produce liver tumours in laboratory rodents, and there is also indirect evidence that microcystins may promote tumours in humans from DW (Svrcek & Smith, 2004).

Toxicity of hepatotoxins varies markedly, even within the family of MCs variants (Table 2.2). MCs toxicity depends on the degree of methylation of MeAsp and Mdha amino acids, and on the stereoisomers of the Adda chain (Svrcek & Smith, 2004). MCs are the most found of the hepatotoxins class. MCs have about 60 variations, MC-LR being the most famous. Knowledge regarding MCs absorption via the gastro-intestinal tract is not extensive because the toxicokinetic studies in which MCs are studied by oral contact are very few. Animal studies using the intravenous or intraperitoneal track show that MCs accumulate mainly in the liver; 50 to 70% of the dose administered is found in this organ (Duy et al., 2000; Health Canada, 2002).

Table 2.1: List of cyanotoxin types and producer organisms (Adapted from Svrcek & Smith, 2004)

Toxin group *(Type)*	**Producer CB genera**	**Toxic** *(adj. toxin = toxic)*	**DW quality and public health significance**
Cyclic peptides			
Microcystins (MCs): Microcystin-LR Microcystin-RR Microcystin-YR Microcystin-LA Microcystin-LW Microcystin-LF	*Anabaena* sp., *Anabaenopsis* sp., *Aphanocapsa* sp., *Hapalosiphon* sp., *Microcystis* sp., *Microcystis aeruginosa*, *Nostoc* sp., *Oscillatoria* sp.	Hepatotoxic	• Hepatoenteritis, • Acute toxicity unlikely in large water supplies, • Chronic liver damage with chronic exposure, • Tumor growth promotion, The relationship between the tumor growth promotion properties of these toxins and carcinogenicity needs to be determined
Nodularins	*Nodularia spumigena* (mainly brackish water)	Hepatotoxic	• As for MCs, • Nodularia is not found in reservoirs ; only blooms in estuarine lakes
Alkaloids			
Neurotoxic alkaloids			
Anatoxin-a	*Anabaena* sp., *Aphanizomenon* sp., *Oscillatoria* sp.	Neurotoxic	
Anatoxin-a(S)	*Anabaena* sp., *Oscillatoria* sp.	Neurotoxic	
Saxitoxins (STXs)*: Saxitoxin (STX) Gonyautoxins (GTX 1, 2, 3, 4, 5 & 6) C-toxins (C1, 2, 3 & 4)	*Anabaena* sp., *Anabaena circinalis*, *Aphanizomenon* sp., *Cylindrospermopsis* sp., *Lyngbya* sp.	Neurotoxic	• Acute poisoning results in death by paralysis and respiratory failure • Acute toxicity only at very high cell densities • No known effects from chronic
Cytotoxic alkaloids			
Cylindrospermopsin (CYN)	*Anabaena* sp., *Aphanizomenon* sp., *Cylindrospermopsis* sp., *Umezakia* sp., *Cylindrospermopsis raciborskii*	Cytotoxic, Hepatotoxic, Neurotoxic, Genotoxic	• Liver damage • Gastrointestinal tract damage
Dermatotoxic alkaloids	Marine CB		
Aplysiatoxin	*Lyngbya* sp., *Schizothrix* sp., Oscillatoria sp.	Dermatotoxic	
Debromoaplysiatoxin	*Lyngbya* sp., *Schizothrix* sp., *Oscillatoria* sp.	Dermatotoxic	
Lyngbyatoxin-a	*Lyngbya* sp.	Dermatotoxic	• Oral and gastrointestinal inflammation
Lipopolysaccharides (LPS)	All (Most CB)	Endotoxic	• Potentially irritates any exposed tissue (Skin, eye irritation; Skin rashes), respiratory allergy, and gastrointestinal disorders • Possible significant for water supply in relation to bathing

* Also known as Paralytic Shellfish Poison (PSP) or Paralytic Shellfish Toxin (PST)

Table 2.2: Acute toxicity of various cyanotoxins (adapted from Codd et al., 2005; and Codd et al., 2005; Hitzfeld et al., 2000; Svrcek & Smith, 2004)

Name	**LD_{50}*** (i.p. mouse µg/kg body weight)
Hepatotoxins	
Microcystins:	
MC-LR	50
MC-LA	50
MC-YR	70
MC-RR	600
[D-Asp3]microcystin-LR	50 to 300
[D-Asp3]microcystin-RR	250
[Dha7]microcystin-LR	250
[(6Z)-Adda]microcystin-LR	>1200
[(6Z)-Adda]microcystin-RR	>1200
Nodularins	30 to 50
Cylindrospermopsin (hepatotoxic in pure form)	200 to 2100
Neurotoxins	
Anatoxin-a and homoanatoxin-a	200 to 250
Anatoxin-a(S)	20 to 40
Saxitoxins (STXs)	10 to 30

* LD_{50} is the dose of toxins that is lethal to 50% of the test population.

MCs also present a hazard for patients on dialysis, in that the maximum allowable concentration (MAC) may not provide them with adequate protection (Institut National de Santé Publique du Québec (INSPQ), 2006). For example, in Brazil, 50% of dialysis patients in a specific clinic died as a result of using microcystin-contaminated water for their dialysis. This case was the first real evidence of the hepatotoxicity of MCs for humans, as they were detected in the water used by the victims.

MCs toxicity in mice was studied in three phases (Fawell et al., 1999): (1) an acute toxicity study as a result of evidence of intraperitoneal bolus, (2) an oral toxicity study on the development of the fetus, and (3) a subchronic toxicity study over 13 weeks. The acute toxicity study was also conducted on rats. The acute toxicity study identified an intraperitoneal LD_{50} level between 50 and 158 µg/kg in mice, and an oral LD_{50} level of over 5000 µg/kg for rats and mice. It was determined that the mouse was the most sensitive species. Thereafter, the toxicity development study showed maternal toxicity at a dose of 2000 µg/kg/day, but there was no dose-dependent effect among the offspring. Thus, the no observed adverse effect level (NOAEL) was set at 600 µg/kg/day. During the subchronic toxicity study, the mice were exposed to concentrations of 40,

200 and 1000 µg/kg/day of MC-LR by gorging. Decreased body weight, but no dose-dependent effect, was observed in treated animals. Light hematologic changes were observed at the highest dose and the groups exposed to 200 µg/kg also showed evidence of changes in their blood chemistry. Finally, histopathological changes in the liver were observed from 200 µg/kg/day. On the basis of these various effects, the lowest NOAEL was determined at 40 µg/kg/day.

At the chronic level, the promotion of precancerous skin tumours was observed in mice (Institut National de Santé Publique du Québec (INSPQ), 2006). However, there is little data for human effects. Thus, the carcinogen classification (IIIB) is determined on the basis of limited evidence in animals and inadequate data in humans (Health Canada, 2002).

Most neurotoxins are acute-acting, that is, they act immediately with a very small dose; hence, chronic exposure does not generally occur. Symptoms of exposure to anatoxin-a include staggering, muscle twitching and gasping in animals, opisthotonus in birds (head and neck stretched backwards along the back), and rapid death by respiratory arrest. Death due to respiratory arrest occurs within minutes or hours, depending on species, dosage, and prior food consumption. anatoxin-a and anatoxin(S) both interfere with the acetylcholine–acetylcholinesterase coordination of the nervous system, disrupting communication between neurons and muscle cells, albeit somewhat differently. Whereas anatoxin-a attaches to receptor molecules and cannot be degraded by acetylcholinesterase, anatoxin-a(S) inhibits acetylcholinesterase from degrading acetylcholine; both cause muscle overstimulation, inducing twitching and cramping, followed by fatigue and paralysis. If respiratory muscles are affected, the animal may suffer convulsions (from lack of oxygen to the brain) and die of suffocation. Anatoxin-a(S) has the additional symptom of excessive salivation. It is interesting to note that as it is a naturally-occurring organic phosphate, anatoxin-a(S) actually functions much like synthetic organophosphate insecticides. The fast-acting neurotoxins STX and neosaxitoxin again disrupt the nervous system, but do so by preventing acetylcholine from ever being released by neurons (Svrcek & Smith, 2004).

The increasing frequency and intensity of cyanobacterial proliferation leading to neurotoxin and hepatotoxin production is a universal problem (Carmichael, 1992; Carmichael, 1994; Chorus & Bartram, 1999; Fawell et al., 1993; Svrcek & Smith, 2004). The distribution of cyanobacterial proliferation records associated with cyanotoxins on different continents and records of the

greatest human health problems caused by CB and their toxins are presented in Appendix D. The main toxins of interest are microcystins (MCs), Anatoxin-a, cylindrospermopsin (CYN) and STXs (Charlton et al., 2001; Duy et al., 2000; Li, Carmichael et al., 2001; Shaw et al., 2000; Shaw et al., 2001). Meanwhile, the MCs analogues are the most commonly reported of the algal toxins world-wide (The Cooperative Research Centre for Water Quality and Treatment (Australia), 2007).

The presence of MCs was monitored under a surveillance plan covering a great number of surface source waters and treated waters in France between 2002 and 2004. In the case of raw water, the concentration of MCs in 88% of samples was under 3 µg/L with more than 63% under 0.16 µg/L; 8% of samples were between 3 and 15 µg/L; and only 4% of samples were over 15µg/L (AFSSA-AFSSET, 2006).

CB blooms are common throughout New York state surface waters, and in recent years, several animal fatalities have occurred due to cyanotoxins (Boyer et al., 2001). These include dog deaths in 1999 due to anatoxin-a and in 2000 due to MC toxicity in Lake Champlain, and dog and water fowl deaths in Lake Neahtawanta in 2004 due to MC. Meanwhile, cyanobacterial blooms routinely occur near the major drinking water (DW) facilities‟ water intakes located on Lake Erie, Lake Ontario and Lake Champlain (Boyer, 2007; Pemberton et al., 2007). In another study, the MCs class of cyanotoxins was found in 33 American water supplies, but the MCs concentration exceeded 1 µg/L in only 7% of samples (Haddix et al., 2007). In a North American study of 45 cities, MC production was resulted from 80% of recorded algal growth cases (Carmichael, Azevedo et al., 2001). MCs and anatoxins were also detected in northern regions including some in Canada (Chorus and Bartram, 1999; Hrudey et al., 1994; Karner et al., 2001; Svrcek & Smith, 2004).

The presence of MC-LR and traces of anatoxins was detected in raw and treated water in Canada (Gurney & Jones, 1997; Jones, 1996; Jones et al., 1998; Robert, Tremblay et al., 2005). Robert, Tremblay et al. (2005) have reported the presence of elevated counts of CB and have also detected several cyanotoxins in Quebec (Canada) surface water. The Quebec MDDEP DW quality monitoring program studied the presence of MCs and anatoxins in six DWTPs from 2001 to 2003 (Robert, Tremblay et al., 2005). The MCs studied were MC-LR, MC-RR and MC-YR. The studied DWTP were those in Plessisville and Daveluyville on the Bécancour River, in Saint-

Hyacinthe, Farnham and Saint-Damase on the Yamaska River, and Bedford in Missisquoi Bay. The three studied MCs were detected in the raw water and the maximum concentration of MC-LR had a measured value of 3.5 µg/L. In the treated water, most of the analysis results were within the detection limit, and the maximum concentration of MC-LR was 0.043 µg/L (Robert, Tremblay et al., 2005).

In Missisquoi Bay (the Canadian side of Lake Champlain), Lake Waterloo and Lake Boivin to the North Yamaska River, cyanobacterial proliferation had continuously been detected during the summer and fall period of 2004-2006 (MDDEP, 2007). Missisquoi Bay is the source of DW for the Saint-Armand, Philipsburg and Bedford municipalities, and many recreational activity centers are situated in this area (e.g. Venise-en-Québec). The North Yamaska River is the DW source for the municipality of Waterloo and Granby, and Lake Boivin and Waterloo are also recreational activity centers in Montérégie (South east of Quebec Province). In 2007, as far as November, 181 lakes and rivers in Québec were affected by cyanobacterial proliferation, suggesting an increased predominance (Ministère de la Santé et des Services Sociaux (MSSS), 2007; Robert, 2007). High concentrations of blue-green algal bloom serve as a signal of a higher probability for toxins occurrence in water sources. Considering the local nature of cyanobacterial proliferation, the possible presence of CB and cyanotoxins at the water intake of drinking water treatment plants (DWTPs) during a source water bloom must be studied for Quebec source waters.

The spatial and temporal distributions of cyanobacterial bloom events in water bodies are local and transitory. Light wind drives cyanobacterial colonies to leeward shores and bays where they form scums (The Cooperative Research Centre for Water Quality and Treatment (Australia), 2004). The global climate change effects, eutrophication of water bodies, and increased water temperatures (over 20^{o}C) have increased the frequency and duration of cyanobacterial blooms (Wiedner et al., 2007). More studies are needed to determine if the spatial distribution of CB has an impact on their public health risk.

2.1.2 Monitoring methods for CB and their associated toxins

Sophisticated monitoring and analytical methods are needed in order to detect the occurrence of CB. These methods can be classified into two main categories, (1) methods detecting CB cells (Table 2.3) without molecular extraction and (2) those detecting their specific molecules

(pigments) once extracted (Table 2.4). Because toxic CB do not release their toxins at any time, the most prudent approach to detecting them is to monitor the whole cell rather than its toxins. However, once the toxic CB cell disappears, it is possible that their toxins remain (Lavoie et al., 2007). It is therefore necessary to identify rapid methods for detecting CB cells and their toxins.

Of the methods mentioned in Tables 2.3 and 2.4, the methods for "detection of cyanobacterial fluorescence" are more in fitting with the needs of high frequent monitoring plans. It is possible to detect the CB cells by fluorescence without molecule extraction. The detection of pigments is based on analysis of their natural fluorescent properties. "Fluorescence" is the molecular absorption of light energy at a certain wavelength; an emission is reissued almost instantaneously at another wavelength, usually longer. Fluorescent pigments have two specific spectra of light energy: an excitation spectrum and an emission spectrum. Fluorescence of phytoplankton can be measured *in vivo* (in the cells, without extraction of pigments) and *in vitro* (after the pigments have been extracted from the cells). *In vivo* fluorescence (IVF) can quantify photosynthetic organisms in their natural environment (by a profiler or in a water sample) for estimating biomass. It is possible to detect the presence of specific classes of phytoplankton through the spectral signature of their pigment fluorescence (Lavoie et al., 2007).

While fluorescence of eukaryotic algae is effectively excited by blue light (approximately 430–530nm), with an emission peak is around 685nm (fluorescence maximum of Chl*a*), cyanobacterial PC is excited in the orange and red parts of the spectrum (approximately 590–630nm) and its emission maximum lies around 650nm. Although fluorescence is a process partially affected by certain external factors such as light conditions, nutrient availability, cell history, etc., it has been shown that it is a very valuable tool for screening analyses of phytoplankton assemblages and for the selective detection and quantification of PC containing CB (Gregor et al., 2007).

Detection methods based on fluorescent pigments are faster than conventional microscopy, but still require regular validation by microscope. Furthermore, fluorescence cannot help identify a particular species, but rather only a group of algae with similar pigments. In fact, the two methods are complementary. When the species present in a particular lake or reservoir are known (by microscopy), it is possible to track their development and spatial distribution with the help of fluorescence tools (Newcombe, 2009).

In order to be able to track the evolution of phytoplankton in real time, one must use an in-situ detection method. The development of the *in vivo* fluorescence (IVF) (Table 2.3) detection method was a major step forward for in-situ detection of phytoplankton. Because IVF detection does not require sophisticated manipulation, it is an ideal method to carry out profiling and monitoring dynamic population of phytoplankton over long periods. IVF can measure changes in CB biomass based on the fluorescence of their particular pigment, which is used to determine whether further sampling should be conducted. This approach is even more relevant for CB that have the ability to move quickly in the water column, allowing them to seek out nutrients in the hypolimnion and gain access to light in the epilimion, hence maximizing their growth. For example, the migration rate of large colonies of *Microcystis aeruginosa* can vary from 5 to 75 meters per day (The Cooperative Research Centre for Water Quality and Treatment (Australia), 2004). It is recommended to use of IVF detection methods for phytoplankton ecology studies and for the routine analysis of potentially toxic CB species in water tanks (Gregor et al., 2007). For these reasons, the detection of CB by IVF can be a wise choice for water authorities.

It is vital to remember that the measurement of chlorophyll and PC by IVF methods does not provide the same reliability as lab-scale analysis, in terms of counting and identification of CB (Cagnard et al., 2006).

In order to assess the significance of cyanotoxins, reliable and robust analytical methods for various toxins are needed. The presence of MCs and nodularins is determined using high performance liquid chromatography (HPLC) with photodiode array (PDA) detection (Sangolkar et al., 2006). This technique permits the quantitative determination of MCs and nodularins and provides a degree of identity confirmation through the ability to produce absorption spectra of the components. This, however, will suggest that MCs and nodularins are present, with a detection limits of 20-50 ng/L, but will not determine the structure of individual toxins (AFSSA-AFSSET, 2006; Nicholson et al., 2007).

Table 2.3: Measurement methods detecting cyanobacterial cells without molecular extraction (Cagnard et al., 2006; Blais, 2007; Gregor et al., 2007; Lavoie et al., 2007)

Measurement method	Description	Advantage	Disadvantage
Visual detection	Visual observation of cyanobacterial presence without any instrument or analysis.	An instantaneous method.	- Detection is possible: o while high biomass is already occurred, o when the cyanobacteria are migrated to the surface forming green scum (which is not the case for all species). - Detection depends highly on the experience of the observer.
Inverse microscopy (Taxonomy)	Cyanobacterium cells are counted based on morphological characters of cells. The cells of cyanobacteria can be preserved in Lugol or Glutharaldehyde. An inverse microscope with an objection of at least 20x effectively allows quantifying the diversity and relative abundance of species of phytoplankton of a water sample.	- Considered the best method. - Direct cell count.	- Requires considerable time and a high level of experiment. - In some cases, the morphological characters are not specific enough to differentiate toxic strains of non-toxic strains. CB are sometimes just as hard to identify.
Detection of cyanobacterial fluorescence:			
Epifluorescence microscopy	Cells are counted based on the addition of dyes which highlight the cyanobacteria possess the PC* using special filters.	Facilitates the count of low concentrations (0.1 to 10 cells/mL).	Highly sophisticated.
Flow cytometry combined with epifluorescence microscopy	Based on the fluorescent properties of chlorophyll pigments and phycobiliprotein. The cells, activated one after the other in front of a laser beam, are characterized by their size and their fluorescent properties.	- An in vivo robust method for the detection of pigments. - Allows differentiating phytoplankton subpopulations.	- Long delays between sampling and achievement of results. - Time-consuming approach and requires unique expertise with expensive equipments.
In vivo fluorescence (IVF):			
IVF fluorimetre measuring each sample individually	Enables the analysis of water samples collected individually. Requires a sample bottle to collect water which is then placed into the instrument.	- Lightweight - Easy to handle, - Sensitive and relatively inexpensive (2000$CAN).	It is not appropriate for the rapid analysis of numerous samples.
IVF fluorimetre with continual circulation (flow-through)	A pump moves water to the fluorimetre that remains on board the vessel or installed at the entrance of a drinking water treatment plant.	- Detect real time variations in the population of cyanobacteria. - Particularly useful to establish an automated tracking system of water quality.	- A correction for the transit time of the sample through the pipe must be applied to the series of measured values. - The air bubbles in the pipe can be problematic.

Table 2.3 (Continue): Measurement methods detecting cyanobacterial cells without molecular extraction (Cagnard et al., 2006; Blais, 2007; Gregor et al., 2007; Lavoie et al., 2007)

Measurement method	Description	Advantage	Disadvantage
Submersible IVF profiler (YSI multi probe)	Direct measurement of the fluorescence of the chlorophyll and PC* in the living cyanobacterial cells using on-line sensors installed on multiparameter probe. They do not provide quantitative pigment concentration data, but rather supply relative data on the biomass of cyanobacteria.	- Profiling and monitoring dynamic population of phytoplankton over long periods. - Particularly suitable for the monitoring of cyanobacteria on a wide stretch of water, where the spatial heterogeneity of cyanobacteria becomes a major problem. - A direct indication of the evolution of the biomass. - A direct indication of the intensity of the blooms, even if the correlations are not constant during special period like the domination of filamentous cyanobacteria.	Interference due to: - water turbidity, - the presence of dissolved organic matter, - variations in pigments concentration contained in cells over temporal scale.
Remote sensing	Specific detector of pigment fluorescence is carried by satellites, air planes or helicopters.	It covers a vast area over water body.	- Satellite photos are rather expensive. - High frequent flight plans is very costly. - Clouds limit the satellite view. - Poor density blooms are not detectable. - Bad weather conditions cancel flight plans.

Table 2.4: Measurement methods detecting specific molecules of CB once extracted (AFSSA-AFSSET, 2006; Chorus & Bartram, 1999)

Measurement methods	Description	Advantages	Disadvantages
Chl*a* dosage measurement	- Determination of cyanobacterial biomass measuring Chl a, considering the correlation between the quantity of Chl a and cyanobacteria. - Chl a measurement using: (1) Spectrophotometer after extraction by acetone, or (2) HPLC.	- Requires relatively simple laboratory equipment: filtration apparatus, centrifuge and spectrophotometer. - Less time consuming than microscopic method	- When used with mixed phytoplankton populations (cyanobacteria and other species), it gives an overestimation of cyanobacterial biomass. - Microscopic estimations of the relative share of cyanobacterial cells among the total phytoplankton is needed. - Less specific and less precise than microscopic method. - Problems associated with HPLC method development.
PC measurement	- Determination of concentration of PC (µg/ml) as the cyanobacterial specific pigment. - Once extracted PC concentration is measured using: (1) Spectrophotometer, (2) Fluorimetre, and (3) HPLC.		- The separation is time consuming and requires numerous purification steps, some of them involving extreme experimental conditions

Combining HPLC with mass spectrometry (MS) makes for a more specific analysis as long as the particular variants can be identified based on their mass spectral data, and analytical standards are available. The detection limit of this method is 10-100 ng/L (AFSSA-AFSSET, 2006). LC/MS/MS offers a further degree of sophistication in that it is generally possible to identify MCs based on their mass spectral data, and this should be further developed as an analytical tool. However, these instrumental methods, especially those utilising mass spectrometry, are relatively expensive and require highly skilled personnel. Despite their relatively low detection limits, their installation methods and instrument preparation are very complex and problematic (Nicholson et al., 2007).

The other main toxin detection methods are biochemical. These methods are based on immunoassay techniques, enzyme-linked immunosorbent assays (ELISA), or inhibition of protein phosphatises enzymes (Sangolkar et al. 2006). These methods are relatively simple and can be easily implemented by laboratories with limited resources. These assays are less time-consuming than the instrumental methods, but do not differentiate individual MCs and simply detect their presence (e.g. 100% detection of MC-LR, 87% detection of MC-RR, 48% detection of MC-YR). The detection limit of the ELISA kits when used for MCs and nodularins is 0.1 to 5 ppb (Fischer et al., 2001; Nicholson et al., 2007). While the ELISA kit method can detect a range of MCs, there is still some concern regarding cross-reactivity. Because of its sensitivity and simplicity, this method is highly attractive for analyzing MCs (Hawkins et al., 2005).

In order to overcome the problems posed by the many variants of MCs with differing toxicities and cross reactivity, it is possible to monitor the toxins via inhibition using the protein phosphatase inhibition assay (PPIA), which provides a crude measure of toxicity. The detection limit of colorimetric PPIA is 100-200 ng/L, and while radiometric PPIA is not yet commercialized it has a detection limit of 20 ng/L (AFSSA-AFSSET, 2006).

Anatoxins are relatively infrequently detected, and methods combining HPLC with LC/MS and LC/MS/MS appear adequate for the few times they are required. Using hydrophilic interaction liquid chromatography (HILIC), anatoxin, STX and CYN analysis can be combined into one procedure, thereby improving efficiency (Nicholson et al., 2007).

HPLC methods with fluorescence detection have been successfully adapted for water samples and can provide accurate results to around 1 µg/L for individual toxins. They are, however,

expensive and time-consuming procedures, because STXs consist of 18 separate compounds that are divided into three chemically different groups. There is significant variation in both the chemistry and toxicity of STXs, and there may be transformations between them. As a consequence, the determination of the complete range of STXs requires three separate analyses. A LC/MS/MS method using HILIC has been developed for separation and detection of STXs in a single run (Nicholson et al., 2007). The LC/MS/MS method for CYP and lipopolysaccharides (LPS) provides highly sensitive and accurate results, but these are complex and time-consuming laboratory methods and they need further modification.

An ELISA method has been developed for STXs in shellfish but is optimised for the toxin profiles more commonly found in marine dinoflagellates. Freshwater dinoflagellates are not known to produce STXs. Although these toxin profiles are different from those found in freshwater CB, an ELISA may find some application for these samples (Nicholson et al., 2007). The detection limit of the ELISA kit for CYP and STXs are 0.05-2.00 ppb and 0.02-0.40 ppb, respectively.

An alternative approach is to measure the toxicity rather than toxin content. Toxins act as sodium channel blockers that can be detected using isolated neuroblastoma cells. Because this method gives the net toxicity of the sample, it has considerable advantages as a screening method that can substantially reduce the amount of HPLC analyses required (Nicholson et al., 2007).

2.1.2.1 Sources of interference in CB fluorescence in vivo monitoring

The rapid estimation of CB in the field is a requirement in the effective management of the risks associated with the presence of cyanotoxins in recreational areas and in water for human consumption. The methods currently available for the detection of CB, e.g. Chl*a* measurement, microscopic counting and taxonomic identification, are not only tedious and costly, but they demand considerable analysis time. Furthermore, microscopic measurements provide information on the number of cells and their taxonomic distribution, but are subject to considerable error, particularly when filaments or colonies are present (Ahn et al., 2007; Lawton et al., 1999; Lawton & Robertson, 1999). Moreover, they are too unwieldy to locate bloom events and monitor their evolution in situ, and are not accessible to municipalities. The *in vivo* measurement of PC by means of submersible probes is an interesting approach to monitor cyanobacterial biomass. This type of measurement has many advantages, among them:

specificity, relative simplicity of operation, measurement speed, portability (well adapted to the establishment of profiles covering a number of points in a source), and easy to operate continuously, all at a relatively modest cost (Asai et al., 2001; Izydorczyk, Tarczynska et al., 2005). However, their use for management purposes raises a number of questions concerning their precision and their specificity in environmental conditions. These questions become particularly pertinent from the point of view of applying this technology to determine what level of contamination should be considered serious enough to trigger a health warning and the need for intervention. In the case of thresholds defined by the numbers or the biomass of CB, the use of in situ PC requires the use of conversion factors to express the relative fluorescence recorded in terms of pigment concentration, the number of CB, or the cyanobacterial biomass. These are complex correlations, and little has been written about them. Moreover, not much is known about the procedures for calibrating and interpreting the data generated by this type of probe, or about how much interest there is in measuring a number of other parameters simultaneously, or how useful it would be to do so.

The *in vivo* probes measure fluorescence using light-emitting diode (LED) sources to estimate the biomass of the main groups making up phytoplankton (green algae, CB, brown algae – diatoms and dinoflagellates and the cryptophytes) by applying the spectral signature differentiation algorithms (Beutler, Wiltshire et al., 2002; Wilhelm, 2003; Parésys et al., 2005). All these tools are based on the targeted excitation of accessory pigments. The photons are then directly or progressively transferred to the Chl*a* terminal receptor, which then emits (Krause & Weiss, 1991). More in-depth analysis of the spectral signatures make it possible to distinguish among groups of CB, like blue and red (Beutler, 2003; Beutler, Wiltshire et al., 2003; Beutler, Wiltshire et al., 2004). The increases in Chl*a* and PC may be related, but these parameters are generally weakly correlated (Ahn et al., 2007; Seppala et al., 2007; Brient et al., 2008).

2.1.2.2 Specificity of the excitation and emission wavelengths

The optimal excitation wavelength and the emission wavelength values vary according to the CB species, and over time for a given species (Seppala et al., 2007). Furthermore, the spectra of the equipment available commercially is not necessarily optimal for all the species present (Beutler, Wiltshire et al., 2003; Seppala et al., 2007). Also, the LED sources used for PC excitation are not always completely specific, and induce a degree of excitation of the chlorophyll contained in the

eukaryotic biomass, although the importance of this interference is not well documented. A suspension of *Scenedesmus quadricausa* measured with a YSI 6131 diode would, for example, lead to a false fluorescence reading of about 77 cells/mL of CB per mg/L of chlorophyll estimated (YSI Incorporated, 2006). The extent of this interference depends on a number of factors, among them the precision with which the emission signal is read, the concentration of the algal biomass, and the type of algal biomass. It is also probable that this bias is partially specific to the optics of the instruments. The use of PC measurements with low densities of CB in the presence of strong concentrations of phytoplankton could lead to overestimation of CB. The levels of Chl*a* in Québec lakes vary considerably (1.4 to 60 mg/L) (Giani et al., 2005). Overestimation at these levels could be sufficient for the misleading warning threshold registration of the cell number. The use of two emission wavelengths could compensate for this interference (Beutler, Wiltshire et al., 2002; Gregor & Marsalek, 2005).

The detailed optical specifications of commercial systems, that define the specificity of these systems, are not always revealed. MINI Tracka, BG-Algae HYDROLAB, ECO Triplet, TriOS miroFlu-blue, bbe FluroProbe, bbe Algae Online Analyser (AOA) fluorometer, Turner Fluorometer and YSI 6131BGA-PC are examples of several commercial models of fluorometric probes available (American Water Works Association (AWWA), 2010; Cagnard et al., 2006; Lavoie et al., 2007). A few recent publications compared the specifications of these probes as published by their manufacturers (Lavoie et al., 2007) and compared their performances in natural waters (Cagnard et al., 2006). The available information on the reliability and precision are limited and contradictory (Cagnard et al., 2006; Collins, 2007; Lavoie et al., 2007). The operability of both the system and the probes is a major factor that must be considered. A systematic study is required to evaluate the commercially available probes‟ response in laboratory conditions, in the field and in DWTP operational situations.

2.1.2.3 Influence of environmental factors on fluorescence measurement

A number of environmental factors influence both the quantity of PC and Chl*a* present per cell and fluorescence expression following excitation by the probe, among the most important being the level of pigment saturation and the turbidity. Manufacturers recognize the interference of turbidity (YSI Incorporated, 2006), and recommend a correction to the PC reading bias (21 cells-equiPC/NTU). However, information on these tests is limited and no measurement with natural

turbidities is available. The impact of turbidity caused by the addition of sand (0.1g/l) would depend on both the number and the size of the particles; small particles (0.1mm) reduce the fluorescence measurement by 12.3%, while larger particles (0.316mm) reduce it by only 5.9% (Brient et al., 2008).

The fluorescence measurement can be influenced directly by the level of sunshine, and indirectly by the level of prior light saturation, and consequently the fluorescence response of the pigments. The short-term removal of a natural or artificial light source (30 minutes) will not affect the fluorescence reading of a *Planktothrix agardhii* suspension, but fluorescence with weak illumination will decreases considerably (Brient et al., 2008).

PC is relatively stable. It is used as a food and cosmetic pigment, and can exist in an abiotic environment for more than a week at a pH of less than 8 (Sarada et al., 1999). The relative persistence in the environment of pigments can be measured by in situ probes following a CB lysis, yet this is not well documented. High PC readings by an in situ probe in the absence of cells have been reported (Brient et al., 2008). The optical changes in a filamentous CB suspension of *Limnothrix Pseudoanabaena* are indicators of the stability of pigments following cell lysis (Simis et al., 2005). According to these authors, the rapid variation of the PC to Chl*a* ratio would be one of the best indicators of the massive lysis of CB. In effect, Chl*a* would persist for several days, while PC would be degraded immediately. The persistence of PC is pertinent to our understanding of the kinetics of the degradation of cyanotoxins, since PC catalyses the photodecomposition of MC-LR (Gajdek et al., 2004).

2.1.2.4 Sources of variability for the conversion of in situ fluorescence measurements into the number of CB

A reliable fluorescence measurement can serve as a basis for the estimation of a cyanobacterial biomass or a number of CB. To arrive at these estimates based on fluorescence measurements, the following factors must be taken into account: (1) the cell content of PC and its variability, and (2) the CB cellular biomass variation over time and space. The pigment content varies as a function of the physiological state of the organisms (Cagnard et al., 2006; Chorus & Bartram, 1999). The PC content per cell is variable and its variation is the origin of the spread of the correlations between fluorescence and the cyanobacterial biomass (Izydorczyk, Tarczynska et al., 2005). PC quantity adjustment to the level of sunshine or light is very fast. Significant

differences in fluorescence per cell between the surface layers (<10m) and deeper layers of water bodies has been observed (Gregor et al., 2007).

The relative fluorescence conversion factor expressed in number of cells must be adjusted to take into account variations in biomass per cell, and in PC content per unit of biomass of the different types and principal species of present CB. Thus, specific conversion factors must be estimated for the principal types of CB prior to the application of *in vivo* probe. The biovolume vary from less than 1 to more than 180 mm^3/cell (Brient et al., 2008). The importance of expressing fluorescence as a function of the corrected biomass has recently been illustrated for two species, *Planktothrix agardhii* and *Lemmermanniella* sp. (biovolumes of 63.87 and 3.12 mm^3 respectively) (Brient et al., 2008). The conversion factors are almost identical when fluorescence is expressed as a function of biovolume. Good correlations (r^2 = 0.73 to 0.78) were observed between *in vivo* PC measurements and CB biovolume at 35 sites in France (Brient et al. 2008). However, the resolution of these correlations is insufficient to permit a precise classification and reflects the challenge of expressing the biovolume well in mixed populations, which vary in time and space. A better resolution has been observed for weaker concentrations (0-12 mg/L of biomass), suggesting that the in situ measurement is a good tool for detecting the development of an efflorescence at an early growth phase (Izydorczyk, Tarczynska et al., 2005).

Because of the variability of fluorescent signals in the natural environment, IVF provides semi-quantitative data on biomass units. The fluorescence signal is converted into information about the biomass by the instrument‟s software (units: cellular abundance or Chl*a*) through empirical relationships established by the company. By contrast, fluorescence is affected by the morphological and physiological characteristics of the species (e.g. cell size, historical conditions of light and nutrients). If greater accuracy in estimating the biomass is desired, it is best to calibrate the fluorescence signal of a profiler for a given body of water (e.g. by analyzing the community using microscopy and HPLC and establishing localised empirical relationships). When CB cells are counted or pigmentary concentration of a sample is quantified, a correction factor for IVF can be developed (Lavoie et al., 2007).

To identify the potential limitations of the probe‟s application, sources of interference and bias need to be quantified, namely: (1) the conversion factor used to estimate the CB biovolume as a function of the probe‟s raw reading unit applicable to environmental field situations; (2)

significance of turbidity interference; (3) the importance of the phytoplankton interference; (4) the necessity to measure Chl*a* simultaneously; (5) the ability of *in vivo* PC measurement to provide reliable estimates at management driven threshold values.

2.2 Treatment methods for CB and their associated toxins

Table 2.5 summarizes the known efficiency of the DW treatment processes against CB cells, cell-bound toxins and dissolved toxins (Carrière et al., 2010; Chorus & Bartram, 1999; Cook & Newcombe, 2002; Hall et al., 2000; Lin et al., 2009; Maatouk et al., 2002; Merel et al., 2010; Montiel & Welté, 1998; Newcombe & Nicholson, 2004; Pietsch et al., 2002; Sano et al., 2011; Svrcek & Smith, 2004; Takaara et al., 2010; Teixeira & Rosa, 2005; Teixeira & Rosa, 2006; The Cooperative Research Centre for Water Quality and Treatment (Australia), 2007; Warren et al., 2010). Also in Table 2.5 the treatment domains that require more investigation are highlighted. Treatment methods in accordance with the hypothesis and objectives of this dissertation are discussed in more details:

- Conventional treatment methods including:
    - Physical pre-treatment.
    - Coagulation, flocculation, clarification (sedimentation/flotation) and filtration.
    - Filtration processes.
- Treatment by chemical oxidation focused on Chlorine.
- Adsorption on activated carbon.

2.2.1 Conventional treatment

Conventional treatment for removal of CB and their associated toxins includes the physical pre-treatment, coagulation, flocculation, separation (decantation/flotation) and filtration processes.

Table 2.5: Techniques for treatment of cyanobacterial intact cells and dissolved cyanotoxins

Treatment process	Cell/toxin	Treatment efficiency
Coagulation/flocculation decantation	Intact cells	Effective for the removal of intracellular toxins. Different cell removal efficiencies observed for different coagulants. Organic matter of certain *M. aeruginosa* inhibits coagulation depending on the coagulant. Coagulation inhabitation observed the growth phase of phase of the cell impacts the removal efficiency. Accumulation of the removed cells in sludge bed seen as a way of isolating toxic cell from the water. Long retention time (clarifiers with permanent sludge blanket) favours the CB cells removal. High cell numbers might increase coagulant demand.
Rapid filtration	Intact cells	Effective for the removal of cell-bound toxins. Cells are not allowed to accumulate on filter for prolonged periods. High cell numbers might cause excessive head loss development, filter clogging, short filter runs and causing an increase of up to 20% of water losses in backwashes.
Slow sand filtration	Intact cells	As for rapid sand filtration, with additional possibility of biological degradation of dissolved toxins
Membrane processes	Intact cells	Very effective for the removal of intracellular toxins. Provided cells are not allowed to accumulate on membrane for prolonged periods
Dissolved air flotation (DAF)	Intact cells	Flotation gives better results than decantation for the separation of low-density floc particles, e.g. CB cells. DAF is found to be more effective than decantation in the removal of CB cells including *Microcystis*. Toxins release during DAF process and sludge treatment is an issue to be considered
Oxidation processes	Intact cells	The efficiency of pre-oxidation depends on cell number, present species, toxins concentration, oxidant dose and contact time. Pre-oxidation induce cell membrane damage and/or lysis and consequent increase in dissolved toxin/taste & odor compounds levels and DBP formation. It is also important to study the influence of water matrix background on DBP formation compared to CB cell related materials (?). Pre-oxidation of cells will prevent process disturbances in DWTP due to presence of CB cells if only the des-advantages are dealt with.
Ozonation	All toxins	Ozonation is effective for all dissolved toxins except the saxitoxins. A residual of at least 0.3 mg/L for 5 minutes will be sufficient. Doses will depend on water quality. O_3 k measured: MC-LR>CYN>ANTX.
Chlorination	All toxins	If a dose of at least 3 mg/L is applied and a residual of 0.5 mg/l is maintained for at least 30 minutes, most MC and cylindrospermopsin (CYN) should be destroyed. MC-LA may require a higher residual. No chlorination kinetics models available for oxidation of STXs using chlorine in different water quality conditions (?).
Chloramination	All toxins	Ineffective
Chlorine dioxide	All toxins	Not effective with doses used in DW treatment
Potassium permanganate	All toxins	Effective for MC, anatoxins and CYN, no data for saxitoxins
Hydrogen peroxide	All toxins	Not effective on its own
UV Radiation	All toxins	Capable of degrading MC-LR and CYN, but only at impractically high doses (1530 to 20000 mJ/cm^2) or in the presence of a catalyst

Table 2.5 (Continue): Techniques for treatment of cyanobacterial intact cells and dissolved cyanotoxins

Treatment process	Cell/toxin	Treatment efficiency
Adsorption – Powder Activated Carbon (PAC)	MCs (except MC-LA) and Anatoxin-a	Steam-activated coal-based PAC is significantly more efficient than wood-based PAC. Highly mesoporous wood-based carbon is more effective than coconut-based carbon. Mesoporous carbons (2-50 μm) are most effective for removing MCs. 60 minutes contact time recommended. Required doses vary with water quality. Water pH plays an important role in removal by PAC.
Adsorption – Granular Activated Carbon (GAC)	MC-LA	High doses required
	CYN	As for most MC
	Saxitoxins	A microporous carbon (steam activated wood, coconut or coal based) 60 minutes contact time recommended effective for the most toxic of the variants
	All toxins	GAC adsorption displays a limited lifetime for all toxins. This can vary between 2 months to more than one year depending on the type of toxin and the water quality
Biological filtration	All toxins	When functioning at the optimum this process can be very effective for the removal of most toxins. However, factors affecting the removal such as biofilm mass and composition, acclimation periods, temperature and water quality cannot be easily controlled
Membrane Processes	All toxins	Depends on membrane pore size distribution; 6 log of cell-bound toxins removal using microfiltration and ultrafiltration; Major filter clogging and risk of toxin release; Water treatment by ultra- and micro-filtration do not have any effect on dissolved toxins except if they are used jointly with PAC; Nanofiltration (NF) and reverse osmosis can retain dissolved toxins; NF removes MC variants (MC-LR, -LY and -LF) and Anatoxin-a regardless of the variations in feed water quality (e.g. NOM), the water recovery rate and the pH values.

2.2.1.1 Physical pre-treatment

Bacterial flocs of CB are smaller than 100µm. Considering their size, screening (one filtration through a screen with a mesh size of 1 to 2 mm), grit removal, aeration, and forced air stripping all have no effect on the retention of CB and dissolved cyanotoxins. Micro-screening at 35 micrometers can retain 40 to 70% of phytoplankton and 10% or less of certain CB, including particular *Microcystis aeruginosa*. This, however, has no impact on dissolved toxins (Chorus & Bartram, 1999).

2.2.1.2 Coagulation, flocculation, clarification (sedimentation/flotation) and filtration

Elimination of 50% to 60% of CB by coagulation, flocculation, sedimentation and filtration has been achieved (Chow et al., 1999; Keijola et al., 1988; Mouchet & Bonnelye, 1998). Meanwhile, the treatment process must be optimized using precise parameters including the measurement of zeta potential or electro kinetic current. Moreover, coagulation is less effective at increasing pH. Therefore, an automatic system would be very useful to continually check the colloids zeta potential in order to optimize coagulant concentration (Bernhardt & Clasen, 1994; Lai et al., 2002).

The flocs are light during the cyanobacterial proliferation period; therefore it is recommended that auxiliaries, e.g. anion polymers, be used for the flocculation process (Bernhardt & Clasen, 1994; Knappe et al., 1998). In certain cases, the use of a flocculating agent containing cationic (AFSSA-AFSSET, 2006) or anionic (Bernhardt & Clasen, 1994; Knappe et al., 1998) polymer allows for a better elimination of the CB. A study by Pietsch et al. (2002) shows that aluminum sulphate was a more efficient flocculating agent than iron salt (4 mg/l of aluminum sulphate and 6 mg/l of iron salt (ferric chloride sulphate) were used).

Regarding sedimentation, horizontal static sedimentation tanks have rather long sludge retention times, whereas vertical static sedimentation tanks show more regulated sludge elimination. In the case of dynamic bed decanters with sludge recycling, sludge retention time must absolutely be taken into account. In this type of treatment, it is the filtration step that plays the most important role by ensuring 90% of total retention (Montgomery, 1985; Hoeger et al., 2002). Direct filtration without preliminary treatment, e.g. flocculation, has been shown not to be effective in

CB retention (Mouchet & Bonnelye, 1998). In the filtration stage, filter clogging is the main problem, causing an increase of up to 20% of water loss during backwashing (Hall et al., 2000).

Many studies have shown that coagulation/sedimentation generally does not cause cell lysis and release of cyanotoxin (Montgomery, 1985; Chow et al., 1998; Chow et al., 1999; Drikas et al. 2001). However, Pietsch et al. (2002) reported significant toxin release by coagulation and filtration. Hydraulic stress (turbulence) caused by flocculation/filtration and pressure gradients in pipes and filters have a destabilising effect on cyanobacterial cells (Pietsch et al. 2002). The stability of cyanobacterial cells varies according to their growth phase. Depending on their growth phase, flocculation/filtration can lead to toxin release (Pietsch et al. 2002). However, if the sludge is preserved more than 1 to 2 days, lysis can occur. Up to 100% of cyanotoxins release was observed within 48 hours. The lysis of CB retained on the filters might happen after 24 to 48 hours of retention, which also implies that good management of the filters maintenance process is paramount (Lepisto et al. 1994; Chorus & Bartram, 1999). Furthermore, the available information on the fate of CB cells and potential toxin release during conventional treatment processes is contradictory with little information available on full scale DWTPs. Cell lysis during coagulation, flocculation, sedimentation and filtration is a potential problem and a source of real concern in cyanobacterial removal. Thus more pilot scale studies are needed to study this treatment issue.

The conventional treatment processes have no impact on dissolved cyanotoxin removal from water (AFSSA-AFSSET, 2006). Biodegradation in fast filters (with processing times of less than 10 minutes) is too insignificant to be taken into account. Only the addition of powdered activated carbon (PAC) allows for their elimination, although it is possible to effectively remove cell-bound toxins during the coagulation/sand filtration phase (Rapala et al., 2006).

Sedimentation can be replaced by flotation in the separation stage of treatment (the treatment process includes coagulation, flocculation, flotation and filtration). Dissolved air flotation (DAF) is the most commonly-used flotation process. It is reported that flotation gives better results than sedimentation for the separation of low-density floc particles (Montiel & Welté, 1998). Furthermore, air flotation is found to be more effective than decantation in the removal of *Microcystis* sp. cells (Chorus & Bartram, 1999). The performance of this process depends on the type of CB present: it is possible to obtain 40 to 80% removal of *Microcystis aeruginosa*, 90 to

100% for *Anabaena* sp. and only 30% for *Planktothrix* sp. (AFSSA-AFSSET, 2006; Mouchet & Bonnelye, 1998). It is unlikely that flotation is more efficient than decantation for the removal of dissolved toxins. However, the continuous elimination of sludge avoids CB lysis (Chorus & Bartram, 1999). The complete removal of *Microcystis aeruginosa* biomass and associated MC was observed in DAF treatment followed by nanofiltration (Teixeira & Rosa, 2006). Ozone-flotation is an alternative to flotation that combines the oxidizing action of ozone in pre-treatment with the physical action of flotation. However, the risk of toxin release due to cell damage under ozonation is still present.

2.2.1.3 Filtration processes

In general, slow filtration gives satisfactory results for the retention of algae and CB in particular (Mouchet & Bonnelye, 1998; Hitzfeld et al., 2000). However, the risk of toxin release should not be neglected. It has been reported that the following toxins have been retained over the filter: up to 80% of toxic *Microcystis* sp., 30 to 65% of toxic *Planktothrix* sp., and 70% of toxic *Anabaena* sp. (Keijola et al., 1988). The major phenomena in play are bioadsorption and biodegradation, which requires an adaptation period of several weeks. After this period, biodegradation occurs within one day, which explains the better results of that stage.

Bank filtration is a natural surface water treatment process that consists of creating a well in alluvial groundwater, which is then related to a zone of depression by pumping, and recharged on a permanent basis by the river or the lake water. Bank filtration has already proven its good performance in the reduction of odorous CB metabolites. These metabolites are responsible for undesirable taste in DW and the retention of turbidity (Chorus & Bartram, 1999). The bank filtration process has been identified as being a good extra-cellular cyanotoxin retention method (Chorus & Bartram, 1999; Miller et al. 2001). Half-life times vary from 4 to 7 days. Two phases are to be taken into account: adsorption onto the sediments, and abiotic decomposition and/or biodegradation (AFSSA-AFSSET, 2006).

Cyanobacterial cell lysis and toxin release caused either directly by treatment process or indirectly because of sludge age or retention is the main point of concern in conventional treatment processes, and needs to be studied for local treatment facilities that are at risk of having cyanobacterial proliferation in their source water.

2.2.2 Treatment by chemical oxidation focused on chlorine

The purpose of treatment by oxidation is to reduce the microbiological risk via the elimination of micro-organisms. Treatment of CB and their associated toxins by chemical oxidation includes: chlorine and chlorine dioxide, ozone, and potassium permanganate. Meanwhile, the oxidation of CB and their associated toxins could be studied as a pre-treatment function or in a chemical disinfection stage. The doses and type of oxidant required for treatment depends on the type of toxin and the water quality. This dissertation is focused on oxidation of potentially toxic CB cells and their associated toxins using chlorine (pre- and post-oxidation).

It is possible to classify the studies on oxidation cyanotoxins in two categories based on the applicability of their results. The studies in the first group focus mainly on the percent oxidation of a toxin by a given oxidant dose or the residual oxidant level required for complete toxin oxidation (Hoeger et al., 2002; Rositano et al., 2001). These studies are valuable for specific sites with challenging water quality (e.g. pH and DOC) and treatment issues (e.g. the application point of the oxidant in the treatment train).The results often depend on oxidant consumption by the natural water matrix and cannot be applied to other waters.

The studies in the second group compare oxidants on a quantitative basis, and their ability to oxidize cyanotoxins in their dissolved form during drinking-water treatment (Acero et al., 2005; Rodriguez et al., 2007). A kinetic model was applied to predict the elimination of three toxins (microcystin-LR, cylindrospermopsin and anatoxin) in natural waters by ozone, chlorine and permanganate (Daly et al., 2007; Rodriguez, Majado et al., 2007; Rodriguez, Onstad et al., 2007; Rodriguez, Sordo et al., 2007). In this model, the apparent rate constants for toxin oxidation and the decreasing concentration of oxidizing agent in the natural water must be known. The oxidant contact time (CT) is defined as the integral of the oxidant concentration over the reaction time ($CT = \int^{t}_{0} [\text{oxidant}]\, dt$). Using the CT values and the rate constants, the concentration of toxin can be calculated thus:

$$[\text{toxin}] = [\text{toxin}]_0 \, e^{(-kCT)} \qquad \text{(Eq. 2.1)}$$

where k is the second-order rate constant for the reaction of the oxidant with the toxin under consideration at the pH of the water. The rate constants are independent of toxin and oxidant concentrations, and can therefore also be applied to natural waters with different toxin

concentrations. Table 2.6 presents the published apparent constant rates for oxidation of cyanotoxins. However, more studies are needed to determine if the rate constants measured in pure water with high toxin concentrations could be applied to predict the oxidation of dissolved and cell-bound cyanotoxins in natural waters (Table 2.6).

Previous studies concluded that with moderate water temperatures and with pH values under 8, the chlorine dosages required to maintain a residual in the distribution system (0.5 mg/l after 30 minutes of contact time) would be expected to result in oxidation of MCs below the detection limit (Newcombe & Nicholson, 2004). The reactions of MC-LR, MC-LA, MC-RR and MC-YR with chlorine behave according to second-order kinetics over a wide pH range (6 to 9), and both MCs and chlorine reactions are first-order (Acero et al., 2005; Daly et al., 2007; Rodriguez, Onstad et al., 2007). An increase of pH has a negative effect on the MCs degradation rate. The apparent second-order rate constant (k_{app}) for the chlorination of MC-LR at 20^{o}C decreased 98% from pH 5 to 9 (Rodriguez, Onstad et al., 2007). Thus, the oxidation of MC is a pH-related reaction with low pHs favouring the overall process. Acero et al. (2005) estimated that oxidation of MC-LR to below the detection limit at pH 8 requires a chlorine exposure of over 80 mg.min/L. In these circumstances, the formation of chlorine by-products (e.g. THMs and HAAs) must be considered. Acero et al. (2005) demonstrated that the chlorine contact times required to reduce MC-LR concentration to the WHO recommended threshold value of 1 µg/L in batch or plug-flow reactors are comparable to those required for 2-log (99%) inactivation of Giardia cysts, and 10 to 100 times higher than those required for 4 log (99.99%) inactivation of viruses at pH 6 to 9. Therefore, the complete degradation of MCs by chlorine into non-toxic compounds is possible if water pH is maintained below 8 during the disinfection processes (Daly et al., 2007). The order of the microcystin analogues reactivity with chlorine was recently presented as follows: MC-LW≥MC-LF>MC-RR>MC-YR>MC-LA>MC-LR (Ding et al., 2010).

Newcombe et al. (2004) have studied the oxidation of metabolically active cells of *Microcystis aeruginosa* and *Nodularin spumigena* with high levels of toxicity. Chlorination of cells resulted in toxin release and 98% of released cyanotoxins were oxidized. However, the formation of chlorination by-products due to released intracellular organic material can be a limiting factor (Huang et al., 2009). It has been observed that the release of intracellular toxins from chlorinated cells were faster than their oxidation by chlorine (Daly et al., 2007; Lin et al., 2009). Also, it has been demonstrated that it is possible to model the reaction kinetics between chlorine and CB

cells using equation 2.1 (Daly et al., 2007; Lin et al., 2009). However, the available information on the chlorination of different CB cells from natural environments, different cell-bound toxins and DBP formation potential are very limited.

Acero et al., (2005) have evaluated rate constants for the reactions of MC-LR, MC-RR and MC-YR with aqueous chlorine species, hypochlorous acid and hypochlorite. The results indicate that the main reaction at pH 6 to 9 is the reaction between MCs and HOCl. These results support the idea that the main reactive site of MCs with chlorine is the Adda moiety (3-amino-9-methoxy-2, 4, 8-trimethyl-phenyldeca-4(E), 6(E)-dienoic acid) of the MC molecule. Monochloramine, which can be formed during the chlorination of ammonia-containing waters, has no effect on microcystin concentrations.

Table 2.6: Second-order rate constants (k_{appt}) for the chlorine reaction with CB cells and cyanotoxins, and missing information (?)

Toxin	CB	pH	k_{appt} toxin $M^{-1}s^{-1}$	k_{appt} CB cell $M^{-1}s^{-1}$	Reference
MCs	*Microcystis aeruginosa*	8.3-8.6	-	670-1100 laboratory culture	(Lin et al. 2009)
-	*Microcystis aeruginosa*	8.3-8.6	-	70-590 Taiwan bloom	(Lin et al. 2009)
-	*Microcystis aeruginosa*	7.6	-	43.6	(Ding et al. 2010)
MC-LR	*Microcystis aeruginosa*	?	-	Cells in Quebec bloom?	?
MC-LR	*Microcystis aeruginosa*	?	Cell-bound in ultrapure water ?	Cells in ultrapure water & Cl_2 demand per cell ?	?
MC-LR	-	8.0	91.5-33 diss.*	-	(Acero et al. 2005)
MC-LR	-	6.8-7.6	242 diss.		(Daly et al. 2007)
MC-LR	*Microcystis aeruginosa*	7.9	10-96 Cell-bound	760 laboratory culture	(Daly et al. 2007)
MC-LR	-	7.6	55.9 diss.	-	(Ding et al. 2010)
MC-LR	Microcystis aeruginosa	?	Cell-bound + diss. ?	?	?
MC-LA	-	6.8-7.6	172 diss.	-	(Daly et al. 2007)
MC-LA	-	7.6	89.5	-	(Ding et al. 2010)
MC-RR	-	6.1-8.0	130-34 diss.	-	(Acero et al. 2005)
MC-RR	-	7.6	136 diss.	-	(Ding et al. 2010)
MC-LF	-	7.6	204 diss.	-	(Ding et al. 2010)
MC-LW	-	7.6	3320 diss.	-	(Ding et al. 2010)
MC-YR	-	7.6	94 diss.	-	(Ding et al. 2010)
CYN	-	8.0	490 diss.	-	(Rodriguez, Sordo et al. 2007)
CYN	*Cylindrospermopsis* sp.	?	Cell-bound ?	Cells ?	?
-	*Aphanizomenon* sp.	?	-	Cells ?	?
Anatoxin	-	4.0-12.0	< 1	-	(Rodriguez, Sordo et al. 2007)
-	*Anabaena circinalis*	8.3-8.6	-	1400-3400 laboratory culture	(Lin et al. 2009)
STX	-	?	Diss. ?	-	?
STXs	*Anabaena circinalis*	?	Cell-bound ?	Cells ?	?
STXs	*Anabaena circinalis*	?	Cell-bound + diss. ?	Cells ?	?

The reaction of anatoxin with chlorine respects second-order kinetics, and is first-order with regards to toxin and oxidant. The elimination of anatoxins by chlorine is a very slow processes ($k < 1\ M^{-1}s^{-1}$), with chlorination half-life values of over 14 hours. Experiments performed with natural water spiked with anatoxin demonstrated that only 8% of anatoxin was removed by applying a chlorine concentration of 3 mg/L. In addition, a negative consequence of this high chlorine dose is the formation of THMs (Rodriguez, Sordo et al., 2007).

The chlorination of CYN is a pH-related reaction, with its maximum rate at pH 7 (Rodriguez, Sordo et al., 2007). At pHs greater than 7, the chlorine efficacy in CYN oxidation diminishes as the apparent rate constant decreases. Also, according to Senogles et al. (2002), the lowest chlorine dose to achieve 99.8% (almost 3 logs) removal of CYN is at pH 7, while at pH 6 and 8 more chlorine is required. CYN is a dissociating compound that explains the pH-dependency of this reaction. The predominant reaction pathway at the pH range of 4 to 12 is the reaction between HOCl and the dissociated form of CYN (Rodriguez, Sordo et al., 2007).

The chlorination of CYN leads to the formation of 5-Cl-CYN, a non-toxic compound that reacts with chlorine 10–20 times more slowly than does CYN (Rodriguez, Sordo et al., 2007). Experiments performed with natural water spiked with CYN demonstrated that a chlorine dose of 1.5 mg/L was enough to oxidize CYN to below the detection limit. From these results, it can be concluded that it is possible to oxidize dissolved CYN at the chlorination stage of DW treatment processes (Rodriguez, Sordo et al., 2007; Merel, Clément et al. 2010).

The limited information on the chlorination of STX suggests removal of STX by chlorine below the detection limit is possible only at high pH values, e.g. 8 and 9. Moreover, to remove 1 log (90%) and more of GTX2, GTX3, C1 and C2, the pH value must be adjusted to 9. Therefore, pH control is necessary in saxitoxin removal by chlorine (Newcombe & Nicholson, 2004). Meanwhile, at these high pH values, chlorination rates of MC and CYN decrease, so the chlorination of mixed solutions of these toxins seems impossible. The impact of water pH on the chlorination of cyanotoxins requires more study.

Oxidation as a pre-treatment stage is used for raw waters. The oxidants used are: chlorine, chlorine dioxide, ozone, potassium permanganate. What they have in common is their ability to kill CB and cause their lysis. Pre-oxidation, with currently low dosages, is not recommended for the removal of harmful cyanobacteria and their associated toxins. Consequently, there are many

concerns regarding the pre-treatment of toxins using chlorine because of secondary reactions and their harmful by-products (e.g. THMs). Pre-oxidation chlorine doses vary from 0.25 to 2 mg/L and are generally fixed according to ammonia concentration and not the concentration of CB and/or cyanotoxins. A chlorine residual of at least 0.5 mg/L is needed after 30 minutes of contact time in order to observe an effect on MCs, depending on pH. With these amounts, the water‟s chlorine demand must be completely satisfied. This generally leads to the formation of secondary organochlorinated compounds, e.g. THM, which are halogenous derivatives of an acetic acid, acetonitrile. Thus pre-treatments by chlorine are to be avoided when a bloom of CB is present in source water (Daly et al., 2007). However, the DBP formation due to chlorination of CB cells in DWTP operational condition requires more detailed investigation.

It is known that the reaction of chlorine with natural organic material in water forming disinfection by-products is strongly temperature-dependent. Thus, it is possible that at lower temperatures, the rate of reaction with cyanotoxins would be greatly reduced (Newcombe & Nicholson, 2004). The possible effects of temperature on cyanotoxin oxidation and on by-products reactions require further investigation (Drikas et al., 2001). In addition, the apparent rate constant for the reaction of STXs and many other cyanotoxins with different oxidants is unknown. In order to study the applicability of oxidizing agents to different waters contaminated with cyanotoxins, it is suggested that a programme be defined in which a wide range of synthetic waters where DOC concentration, NOM character and alkalinity will be varied systematically and studied. These synthetic results are to be confirmed using water samples from local source waters. A sensitivity analysis can then be undertaken to determine the relative effects of these parameters. The outcome will allow a more confident application of oxidant agents.

2.2.3 Adsorption on activated carbon and biodegradation

Treatment by adsorption on activated carbon is used particularly for the elimination of organic micro-pollutants, including cyanotoxins. Adsorption on activated carbon can be achieved: (1) at the treatment head by using powder activated carbon (PAC) during the coagulation phase, (2) by filtration on granular activated carbon (GAC), or (3) by using reactors where PAC is coupled with retention by membrane, or micro- or ultrafiltration.

Several authors have described the ability of PAC to adsorb cyanotoxins (Bernazeau et al., 1995; Himberg et al., 1989; Ho et al., 2011; Maatouk et al., 2002; Rositano & Nicholson, 1994;). The

doses of PAC and the type of activated carbon required for treatment depend on the type of toxin and on water quality. Water pH plays an important role in removal by PAC. In periods of algal proliferation, the pH level can exceed the conditions of carbon validation (Mouchet& Bonnelye, 1998; Newcombe & Nicholson, 2004).

The efficiency of PAC in the removal of different variants/types of MCs is of the following order: MC-RR>MC-YR>MC-LR>MC-LA (Cook & Newcombe, 2002; Ho et al., 2011). Keeping the same order, steam-activated coal-based PAC is significantly more efficient than wood-based PAC (Cook & Newcombe, 2002). Meanwhile, highly mesoporous wood-based carbon is more effective than coconut-based carbon for absorption of cyanotoxins (Donati et al., 1994; Svrcek & Smith, 2004). In general, mesoporous carbons (2-50 μm) are most effective for MCs removal (Newcombe & Nicholson, 2004). Using PAC with a dose of 11 mg/L, Mouchet et al. (1998) have reported removal of 90% of MCs and anatoxin-a. Meanwhile, the adsorption of MC-LR and MC-LA to PAC was found to be relatively insensitive to the NOM character of water, but varied directly with the DOC of the water (Cook & Newcombe, 2002).

Ho et al. (2011) observed no difference in removal of MCs and CYN using PAC contact times of 30, 45 and 60 minutes. The application of PAC at the raw water step is ideal if there is a sufficient contact time before coagulation. An acceptable compromise is the application of PAC shortly after coagulation to minimize the interference of organic matter, but PAC must be incorporated into the floc to ensure its removal. The accumulation of PAC in the sludge bed of the clarifier has the advantage of increasing the contact time and concentration of PAC. PAC can also be used upstream of micro-filtration or ultra-filtration membranes. In the case of high dosages of PAC, the required backwash frequency could be very high (European Commission, 2005).

Granular activated carbon (GAC) can be used in adsorption mode or in biological mode, and is often preceded by ozone when used in the latter mode. The goal of the use of GAC is the retention and/or the biodegradation of molecules not having reacted with ozone (Hoeger et al., 2002; Lai et al., 2002; Rositano et al., 2001), or the molecules created because of ozone reactions. This stage is regarded as very effective for the retention of toxins, with retention rates of over 90% during the first year of carbon use (Lambert et al., 1996). Considering the typically transient nature of cyanotoxins in raw water, GAC should provide an effective barrier with an

empty bed contact time (EBCT) of 15 minutes or more. In adsorption mode, Newcombe et al. (2004) have demonstrated the breakthrough of MCs after only 1 month of application. In other pilot experiments, carbon lifetime in adsorption columns was from 30 to 45 days (Hart & Stott, 1993). Water quality and, in particular, the quantity of organic matter, play very important roles in toxin removal. For example, the effective retention period will be decreased for carbon used in water with high loading (Hitzfeld et al., 2000). The competitive effect of NOM on GAC adsorption reduces the efficiency of MC-LR removal. Furthermore, low pH values enhance adsorption of MC-LR by GAC (Huang, Cheng et al., 2007).

Studies have described effective biodegradation of MCs by micro-organisms in both source waters and using biological filters (bio-filters) (Bogosian & Bourneuf, 2001; Ho et al., 2006; Ho et al., 2007; Jones, Bourne et al., 1994). In a pilot test with a preliminary dosage of 14 µg/L of MC-LR in raw water, Lambert et al. (1996) observed toxin breakthrough using bio-filtration with 7.5 minutes of contact time, while no breakthrough was detected in a filter with 15 minutes of contact time. Newcombe and Nicholson (2004) showed that a GAC filter in adsorption mode switched to biodegradation mode within 16 days.

The biodegradation of microcystins is fast but bacterial flora requires an adaptation period of some days (Jones & Orr, 1994). In water bodies with a history of algal proliferation, bio-filtration is efficient for microcystin removal from the first moments of their appearance, as long as the required degradation micro-organisms are present (Heresztyn & Nicholson, 1997Rapala et al. 1994). To date, only a few bacterial species of the genus *Sphingomonas* have shown the ability to effectively degrade microcystin. While these organisms are widespread, they are not present in all waters. Degradation by bacteria is not known to produce any harmful by-products. Degradation to below detection level is usually expected to occur within 2-3 weeks in the presence of sufficient numbers of degrading bacteria. An effective biofilter, with the appropriate biomass and microbial community, can reduce high levels of microcystin to below detection level. Genetic methods, such as the polymerase chain reaction (PCR), should allow the screening of water sources and bio-filters to determine whether they contain the bacteria able to degrade microcystin. In situations where the bacteria are not present, it may be possible to artificially seed the degrading bacteria into the system to remove the toxin (The Cooperative Research Centre for Water Quality and Treatment (Australia), 2007). The microbial degradation of dissolved CYN has been confirmed in natural waters with a previous history of toxic

Cylindrospermopsis sp. proliferation. In these water bodies, the degradation of CYN began 3-11 days after their appearance in the water. Once degradation had begun, the CYN was completely removed within 10-33 days. The degradation of CYN in water bodies with no recorded history of toxic *Cylindrospermopsis* sp. blooms began approximately 3 months after their first appearance in water body. Once degradation was detected, only 16-59% CYN removal was observed after 212 days (The Cooperative Research Centre for Water Quality and Treatment (Australia), 2007).

2.2.4 Sludge handling

Permanent elimination of sludge must be considered in conventional treatment processes. Sludge treatment needs particular attention as intracellular toxins might slowly be released during treatment. Thus, water recycling following these treatment steps will be processed in the presence of toxic CB, so it is essential not to underestimate their impact on the natural environment (Chorus & Bartram, 1999). CB, removed by treatment, can undergo cell lysis and potentially release toxins during storage or handling of backwash wastewater and sludge. There is a high risk of significant cell lysis in backwash settlement tanks, sludge thickeners and sludge dewatering by centrifuge or filter press. It is possible to minimise this risk by: (1) avoiding recycling of sludge at times of greatest cyanotoxin risk (although this is not always possible, especially during summer months when water resources are limited), (2) minimising sludge agitation to reduce the potential for cell lysis, (3) minimising sludge storage times prior to thickening and dewatering, (4) minimising sludge retention time in thickeners (European Commission, 2005).

2.3 Management

The surveillance and detection of microcystins in drinking water is becoming more common in many countries worldwide following the recent promulgation of a 1 µg/L guideline level for safe DW supplies set by the World Health Organization (WHO) (Chorus & Bartram, 1999; Hoeger et al., 2002). However, other toxins such as anatoxins, STXs and CYN have also been frequently detected in source water (Hoeger et al., 2004; Molica et al., 2005).

The alert levels set by the Ministère du Développement durable, de l'Environnement et des Parcs (MDDEP) of Quebec and international organizations are based on the potential for the presence of cyanotoxins. The calculation of potential toxicity is derived from the following assumptions:

(1) the toxin content of cell units are quasi-constant; and (2) all cyanobacterial species produce cyanotoxins. Indeed, the CB genetically capable of producing toxins produce relatively the same amount of toxins per unit biomass in each algal bloom event (Giani et al., 2005). However, in an observed algal bloom, only a fraction of the CB population is capable of producing toxins (AFSSA-AFSSET, 2006).

Health-based cyanotoxin exposure guidelines of different water authorities are summarized on Table 2.7. The WHO guideline value (GV) for MC-LR (1 μg/l) was calculated using Eq. 1 (Hudnell, 2006).

$$GV\ (MC\text{-}LR) = (TDI \times BW \times P_{intake}) / (Daily\ consumption)$$

$$= (0.04 \times 60 \times 0.8\ ) / 2 = 0.96 \sim 1 \mu g/L \qquad \text{(Eq. 1.1)}$$

where

- Tolerable daily intake (TDI) is NOAEL (40 μg/kg/day : calculated in 13-week sub-chronic oral mouse dosing study with MC-LR) divided by uncertainty factor of 1000 (10× for intra-species; 10× for inter-species; 10× for limitations of data and lack of data on chronic toxicity and carcinogenicity),
- BW is body weight in kilograms (kg),
- P_{intake} is toxin intake percentage by humans,
- The daily human consumption is in litters.

Table 2.7: Summary of cyanotoxins health-based exposure guidelines/recommended alert levels (MDDEP, 2007; Merel et al., 2010; Newcombe et al., 2010; Svrcek & Smith, 2004)

Cyanotoxin	Organisation/Country	Guideline Value (GV)
MC-LR	South Africa	0.8 µg/L MC-LR
	World Health Organization (WHO), Czech Republic, China, France, Italy, Japan, Korea, New Zealand, Norway, Poland	1.0 µg/L MC-LR
	Brazil, Spain	1.0 µg/L MCs toxicity equivalent
	Australia	1.3 µg/L MC-LR toxicity equivalent
	Québec (Canada)	1.5 µg/L MC-LR toxicity
	Oregon, USA	1.0 µg/l
Cylindrospermopsin	New Zealand	1.0 µg/l
	Brazil	15.0 µg/L
Anatoxins	Québec	3.7 µg/l
Saxitoxins	Australia, Brazil, New Zealand	3.0 µg/L STX toxicity equivalent

Table 2.8: Guideline levels for managing drinking source waters containing cyanobacterial cells (Adapted from Chorus & Bartram, 1999)

Alert level	Density of Cyanobacterial cells	Action
Vigilance	200 cyanobacterial cells/ml	• Non-bloom condition, weekly monitoring
1	2000 cyanobacterial cells/ml or 1 µg/l Chl*a* with a dominance of CB	• Weekly cyanobacterial count • Weekly toxin testing to be initiated in drinking water supplies • Issue advisory notice to public
2	100 000 cyanobacterial cells/ml or 50 µg/l Chl*a* with a dominance of CB	• Weekly cyanobacterial count • Weekly toxin testing in drinking water supplies • More extensive advice to public • Switching to alternative supply should be considered if available

The BW and P_{intake} factors vary depending on the guideline; for instance, in Australia, the BW and P_{intake} are 70 kg and 0.9 respectively, with a guideline value of 1.3 µg MC-LR/L. Based on cyanobacterial cell enumeration and potential cyanotoxin production per cell, guideline levels for bathing and drinking waters are applied by different public health organisations. Table 2.8 present the monitoring strategy recommended by the WHO. Parallel measurement of

cyanobacterial cells and chlorophyll concentration using high-frequency monitoring plans secure the recreational and sanitary use of source waters. Also, Appendix E presents the decision support flow chart for cyanobacterial management in Québec (Canada).

Cyanobacterial blooms routinely occur near the major DW facilities‟ water intakes located on the Great Lakes and Lake Champlain (Boyer, 2007; Pemberton et al., 2007). However, these toxins have not been observed in the related water distribution systems during these studies (Boyer et al., 2001). The sampling method, sampling frequency and sampling scheme all effect efficacy of monitoring strategies in producing data that are representative of environmental events (Zamyadi et al., 2007). A management plan based on a non-representative monitoring strategy leads to over- or underestimation of the CB presence and therefore effects the adjustment of treatment option for DW production. Incidentally, the routine detection of cyanotoxins of relevance to health in source water raises several challenges for water authorities: (1) toxin production is transitory in nature and difficult to predict; (2) the types of toxin produced vary in time and location; (3) several tedious and expensive analytical methods must be used to detect several types of toxins (Codd, Lindsay et al., 2005; Codd, Morrison et al., 2005; Gregor et al., 2007). Moreover, the relation between the CB concentration in water and the probability of the cyanotoxins production is complex.

The health relevance of cyanotoxins, the treatment challenges to remove these compounds and the observed trends of growing predominance of CB in surface water all raise the issue of their presence in water sources that were not previously considered at high risk of cyanotoxin contamination. Furthermore, cyanotoxins including MCs were recently added to the United States Environmental Protection Agency (USEAP) Contaminant Candidate List (CCL). Guidelines for management of CB and their associated toxins are now available in the province of Quebec (Canada) design guides for DWTPs (MDDEP, 2006). Elements of cyanotoxin management plans include:

a) Mandatory guidelines and standards for cyanotoxins in DW

b) Establishment of a plan to monitor CB and their associated toxins

c) Treatment methods for removing cyanotoxins

CHAPTER 3 HYPOTHESES, OBJECTIVES AND RESEARCH APPROACH

This chapter present the objectives, hypotheses and the research approach under this dissertation"s three research themes, (1) monitoring, (2) treatment by oxidation and (3) management. Furthermore, this chapter provides an overview of the articles and how they correspond with the objectives.

3.1 Objectives and hypotheses

The general objective of this Ph.D. project is to study the *in vivo* monitoring of CB and their associated toxins in DW sources and their potential removal in conventional treatment and oxidation. These specific objectives are organised based on the hypotheses. Objectives 1 to 3 cover the monitoring theme of this project. Objectives 4 and 5 are to verify the treatment of CB and their associated toxins using oxidation by chlorine. Objectives 6 to 8 are defined to verify the management theme of this research project. Thus, the specific objectives of this Ph.D. research project are to:

1. Validate the use of online fluorescence probes based on measurements of PC in natural waters.
2. Study the spatial-temporal distribution of CB in Quebec (Canada) water bodies likely of having cyanobacterial proliferation and show the impact of cyanobacterial bloom in water at the water intake.
3. Monitor the concentrations of CB level and their associated toxins in several DW intakes using a high frequency/event based sampling strategy (e.g.: two times per week, sampling when taste and odour are reported, etc.) using a combination of standard enumeration and online methods.
4. Document the efficacy of chlorine to compromise different cyanobacterial species and oxidize a wide range of cyanotoxins in the pre-treatment stage as well as reduce their toxic potential. Determine and integrate the impact of water quality on models predicting the oxidation of selected cyanotoxins by chlorine.

5. Study the release of cell-bound organic material and their contribution to the disinfection by-products precursors‟ pool during direct chlorination of intact cells.
6. Analyse the efficiency of cyanobacterial management strategies to prevent cyanobacterial breakthrough in DWTPs implemented by different water authorities in Australia.
7. Detect the potentially toxic CB and their associated toxins in water samples during cyanobacterial bloom formation using ELISA kits and a multi-toxin LC-MS/MS method.
8. Document the removal of CB and their associated toxins (cell-bound and extracellular) through conventional treatment (coagulation, clarification and filtration) and oxidation (chlorine) in pilot and full-scale plants.

Based on the critical review of literature eight hypotheses were defined to be verified during this Ph.D. research project. The objective of this research are grounded on these hypothesis. Hypotheses 1 to 3 cover the monitoring theme of this project. Hypotheses 4 and 5 are to verify the treatment of CB and their associated toxins using oxidation by chlorine. Hypotheses 6 to 8 are defined to verify the management theme of this research project. The hypotheses that this research project are based on are as follows:

1. The biomass of CB can be estimated using an *in vivo* fluorescence probe in typical environmental water body conditions. Online fluorescence probe measures the fluorescence of PC as specific pigment of CB in fresh waters.
2. Location of cyanobacterial blooms defines its potential impact on the concentration of CB in the water intake. The spatial accumulation of CB varies significantly in a water body and does not necessarily have any influence on the concentration of CB in the water intake.
3. Transient but locally significant cyanobacterial blooms can lead to the presence of cyanotoxins in DW sources and recreational water of southern Québec (Canada). Cyanobacterial blooms and toxin occurrence at these intakes can be detected with a higher frequency or an event based monitoring program.
4. Oxidation by chlorine, at the pre-treatment stage, can compromise cyanobacterial cells, release cell-bound toxins, oxidize cyanotoxins and remove their toxicity; the impact of water quality on the kinetics and efficiency of oxidation by chlorine of both the CB cells and released toxins can be predicted and integrated into predictive modelling.

5. Chlorination of intact CB cells causes formation of DBP, e.g. trihalomethanes (THM), haloacetic acids (HAA) and N-nitrosodimethylamine (NDMA), due to release of cell-bound organic materials.
6. Low frequency monitoring plans recommended in cyanobacterial management strategies provide DWTPs operators with reliable information about the dynamics of their source to adjust the treatment process to prevent cyanobacterial and their associated toxins to accumulate in and breakthrough the treatment plants.
7. Increase of cyanobacterial bloom events over the source and at the water intake is associated with a higher probability of the production (cell-bound) and release (dissolved extra-cellular) of cyanotoxins. The observed concentrations of cyanotoxins are generally significantly lower than those calculated from the content of cell units and an enumeration of cyanobacterial cells.
8. Conventional treatment, carbon adsorption and post-oxidation (with chlorine) constitute effective barriers to remove cell bound cyanotoxins but may not remove all dissolved toxins. The post-oxidation is an efficient barrier for removal of dissolved toxins.

The material and methods of the presented papers and the technical report describe the details of the methodology used during the experiments conducted in this Ph.D. research project. The aim of the rest of this chapter is to present under the three research themes the research approach and general organisation of the five papers and the technical report. Also, this chapter will relate the presented papers/manuscripts with the objectives.

3.2 MONITORING: Validation of *in vivo* fluorescence probes and their application in monitoring DW sources and intakes in Quebec (Canada)

Chapter 4 of this dissertation (manuscript submitted to the JAWWA) covers the first three objectives of the conducted Ph.D. research project on validation and application of *in vivo* fluorescence probes in CB monitoring for DW production purposes. *In vivo* fluorescence probes from three commercial manufacturers, TriOS GmbH (Oldenburg, Germany), biological·biophysical·engineering (bbe), Moldaenke GmbH (Kronshagen, Germany) and Yellow Springs Instrument (YSI, Yellow Springs, Ohio, USA) were used in this study. YSI *in vivo* PC (YSI 606131), Chl*a* (YSI 606025) fluorescence probes and an optic turbidity probe (YSI

606136) were installed on an online YSI 6600 V2-4 water quality multi-probe system. The second *in vivo* PC probe used in this study is a TriOS MicroFlu-blue (TriOS) submergible fluorometer. The bbe FluroProbe (bbe), the third probe studied, is also a submergible spectrofluorometer for algal *in vivo* detection.

To validate the use of online fluorescence probes based on PC measurement, two YSI 6600 V2-4 series multi-probe systems were purchased in 2007. The first season of sampling (summer and fall 2007) was conducted at the water intakes of two DWTPs over Missisquoi Bay and the Yamaska River basin (Figure 3.1). These sites were selected based on limited CB historic data available in Quebec. In 2008, the research on the validation of the probes was expanded under a strategic regrouping project including EPM team, Dr. David F. Bird‟s team from the Université de Quebec a Montreal (UQAM), and the Australian water Quality Center (AWQC) team.

In 2008, with the purchase of another two YSI 6600 V2-4 series multi-probe systems (Dr. Sarah Dorner participation to the FQRNT project) intensive *in vivo* monitoring at the water intake and over the water bodies were added to the field monitoring sites at the Missisquoi Bay and Yamaska River basin. Also, the spatial-temporal distribution of CB in these water bodies within proximity to the water intakes was studied by monitoring the CB proliferation in the lake/reservoirs. All field samplings were coupled with samplings for laboratory taxonomic count and speciation, cyanotoxins analysis and pigments extraction.

Laboratory probes validation were conducted in the EPM (2008 and 2009) and AWQC (2008 and 2010) laboratories using several laboratory cultured CB and eukaryotic algae species as well as different sources of inorganic turbidity. Parallel to all laboratory probe measurements, samples were taken for taxonomic counts, toxin analysis in the case of toxic cultures and pigment extractions. In addition to the YSI probe, the laboratory validations also included TriOS and bbe probes. The TriOS and bbe probes were an in kind contribution from the Centre d‟expertise en analyses environmentales du Québec (CEAEQ) of Quebec‟s Ministry of the Environment and Dr. Isabelle Laurion, from Institut National de la Recherche Scientifique (INRS) Centre Eau Terre Environnement (ETE), respectively. Moreover, a project has been started on the development of the new generation of *in vivo* fluorescence probes in collaboration with Yellow Springs Instrument (YSI, Yellow Springs, Ohio, USA).

In addition to the manuscript presented in Chapter 4 of this dissertation, the conducted research also made possible the publication of another paper as well as the preparation of an additional manuscript:

- N. McQuaid, A. Zamyadi, M. Prévost, D. F. Bird and S. Dorner (2011). Use of *in vivo* phycocyanin fluorescence to monitor potential microcystin-producing cyanobacterial biovolume in a drinking water source. Journal of Environmental Monitoring, Vol. 13, pp: 455-463.
- A. Zamyadi, N. McQuaid, M. Prévost and S. Dorner (will be submitted September 2011). Monitoring of potentially toxic cyanobacteria using an online multi-probe in drinking water sources. Journal of Environmental Monitoring.

In 2009 and 2010 the project continued with monitoring the concentrations of CB level in five monitoring sites (Figure 3.1) including four DW intakes located on (1) MB on the Canadian side of the Lake Champlain, (2) Yamaska River basin, (3) Lake St. Louis, (4) St. Lawrence River (sector of the island of Montreal), and the fifth site Roxton Lake as a recreational water body which is located within the Yamaska River basin. The monitoring plan included a high frequency/event based sampling strategy (e.g. two times per week, sampling when taste and odour are reported, or when bloom events were observed, etc.) using a combination of standard enumeration and online methods.

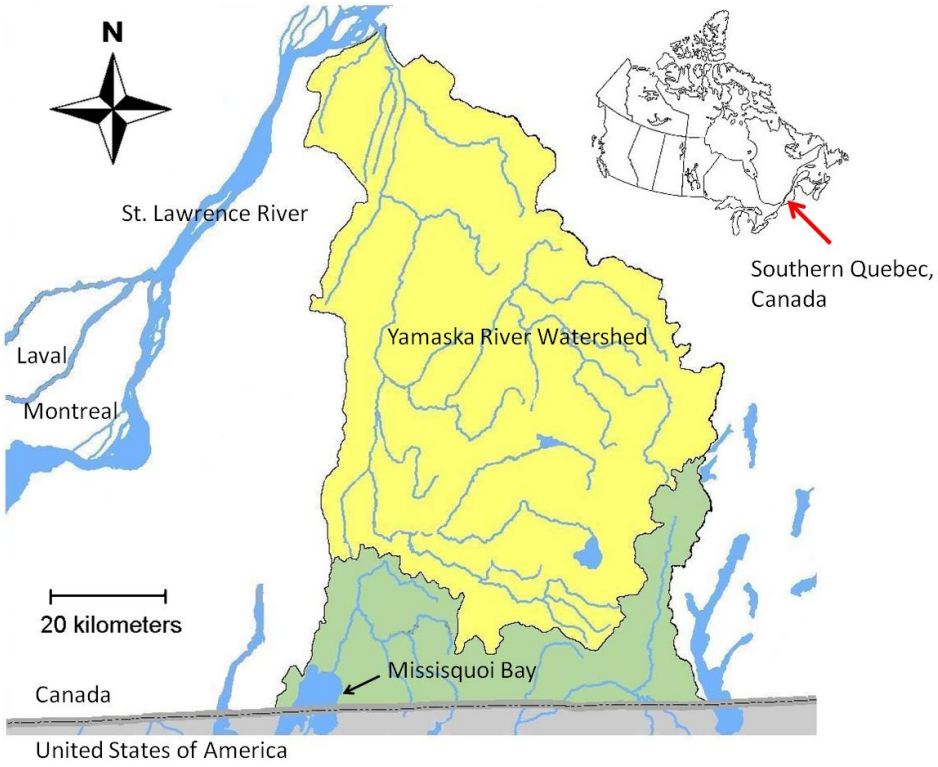

Figure 3.1: The studied sites were located on Missisquoi Bay (Canadian side of Lake Champlain), Yamaska River basin and St. Lawrence River basin sector of the island of Montreal

3.3 TREATMENT BY OXIDATION: Fate of CB cells, their associated toxins and DBP formation during chlorination

Chapters 5, 6 and 7 of this dissertation satisfy the fourth and fifth objectives under the treatment by oxidation theme on the chlorination of cells and their associated toxins for DW production. A comprehensive literature review on the removal of CB cells and their associated toxins was conducted prior to research planning. This literature review helped to uncover the knowledge gaps with regards to the oxidation of potentially toxic CB that required further investigation. Also, the results of this literature review (e.g. published oxidation kinetic model) helped to evaluate the performance of Quebec DWTPs against potential cyanotoxins scenarios in DW water sources and prepare the following publication:

- B. Barbeau, A. Carrière, M. Prévost, A. Zamyadi and P. Chevalier (2008). Changements climatiques au Québec méridional - Analyse de la vulnérabilité des installations québécoises de production d‟eau potable aux cyanobactéries toxiques. Institut national de santé publique

du Québec, 2008, 16 pages. No INSPQ : 867. ISBN imprimé : 978-2-550-54677-1, ISBN PDF : 978-2-550-54678-8

- A. Carrière, M. Prévost, A. Zamyadi, P. Chevalier and B. Barbeau (2010). Vulnerability of Quebec drinking-water treatment plants to cyanotoxins in a climate change context. Journal of Water and Health, © IWA Publishing, Vol. 08.3, pp: 455-465.

Chapter 5 (Paper published in ES&T) presents the novel data on the chlorination of *Anabaena circinalis* cells and their associated STXs in ultrapure and natural water. This chapter included the kinetic models for the chlorination of cells, cell-bound and dissolved STXs. Also, the paper presents a discussion of the integrity of *Anabaena circinalis* cells during chlorination. Furthermore, DBP formation potential of *Anabaena circinalis* during chlorination is presented in this chapter. The focus of this research conducted at the laboratories of AWQC was to carry out the experiments in conditions close to operational conditions in DWTPs. The novel outcomes of this paper demonstrate the need for further investigation of (1) the integrity of different CB species during chlorination, (2) the fate of different cyanotoxins in chlorination and (3) the DBP formation during these chlorination experiments.

The manuscript presented in Chapter 6 (submitted to Water Research) documents the efficacy of chlorine to compromise different cyanobacterial species and oxidize a wide range of cyanotoxins in the pre-treatment stage. Also, this chapter determines and integrates the impact of water quality on models predicting the oxidation of selected cyanotoxins by chlorine and the DBP formation during these experiments. This project was conducted in AWQC laboratories using the raw water of a DWTPs operated by Sydney Water (NSW, Australia) spiked with different CB cells and cyanotoxins.

The manuscript presented in Chapter 7 studied the release of cell-bound organic material from *Microcystis aeruginosa* and their contribution to the DBP precursors‟ pool during direct chlorination of intact cells. A key factor considered during the DBP formation in this section of the study is the long chlorine contact time of 24 hours. Also, in this chapter the novel data on *Microcystis aeruginosa* chlorine demand per cell are presented.

3.4 MANAGMENT: CB blooms and their associated toxins challenge for DW production and efficiency of Australian cyanobacterial management strategies in detection of spatial-temporal distribution of CB

The third research theme of this Ph.D. research project is on the management of CB and their associated toxins for DW production. Australian freshwater sources have a long documented history of CB related issues. Consequently, the Australian research centers (e.g. AWQC) and the water authorities have a vast amount of experience in dealing with CB related issues and their management for DW production.

The Murray-Darling basin is the most populated watershed in Australia with a comprehensive documentation of CB related issues by the Australian water authorities involved with water management in this basin. CB related issues are a relatively new concern for Quebec‟s DW industry. Therefore, in 2010 an industrial visit, funded by the Quebec government, was organised to analyse the efficiency of cyanobacterial management strategies to prevent cyanobacterial breakthrough in DWTPs implemented by different water authorities in Australia. Appendix F presents the outcome of this visit. Also in Appendix F the survey form that was used to document the Australian experiences is presented. The report presented here will cover the sixth objective of this dissertation and helps to verify the sixth hypothesis which falls under the management research theme.

A comprehensive literature review was conducted on the treatment methods for removal of CB and their associated toxins prior to project commencement. The focus of the literature review was to separate the previous published results on treatment option based on the scale of the research: laboratory, pilot or full-scale. The outcome of the papers published in previous chapters demonstrates that appropriate interpretation of laboratory findings to the full-scale conditions is a challenge for application of these findings to operational situations.

The manuscript presented in Chapter 8 (submitted to the Water Research) answers the seventh and eighth objectives. The capability of different conventional treatment processes (coagulation, clarification and filtration) and oxidation (chlorine) for the removal of cell-bound and dissolved cyanotoxins in pilot and full-scale plants were studied by sampling and analyzing water passing through each process. Full scale samplings to document CB removal by conventional treatment

were performed during the summers of 2008, 2009 and 2010. Trigger values of indicators (e.g. observations of green scum) were used to initiate detailed process sampling in treatment plants. These samplings were designed to detect the potentially toxic CB and their associated toxins inside DWTPs during cyanobacterial bloom formation using ELISA kits and a multi-toxin LC-MS/MS method.

This research project was focused on proposing recommendations for best-practice management of CB and their associated toxins in DWTPs in Quebec. Also, this project was aimed to clarify whether modification of existing Quebec guideline on detection, treatment and management of CB and their associated toxins for DW production is required.

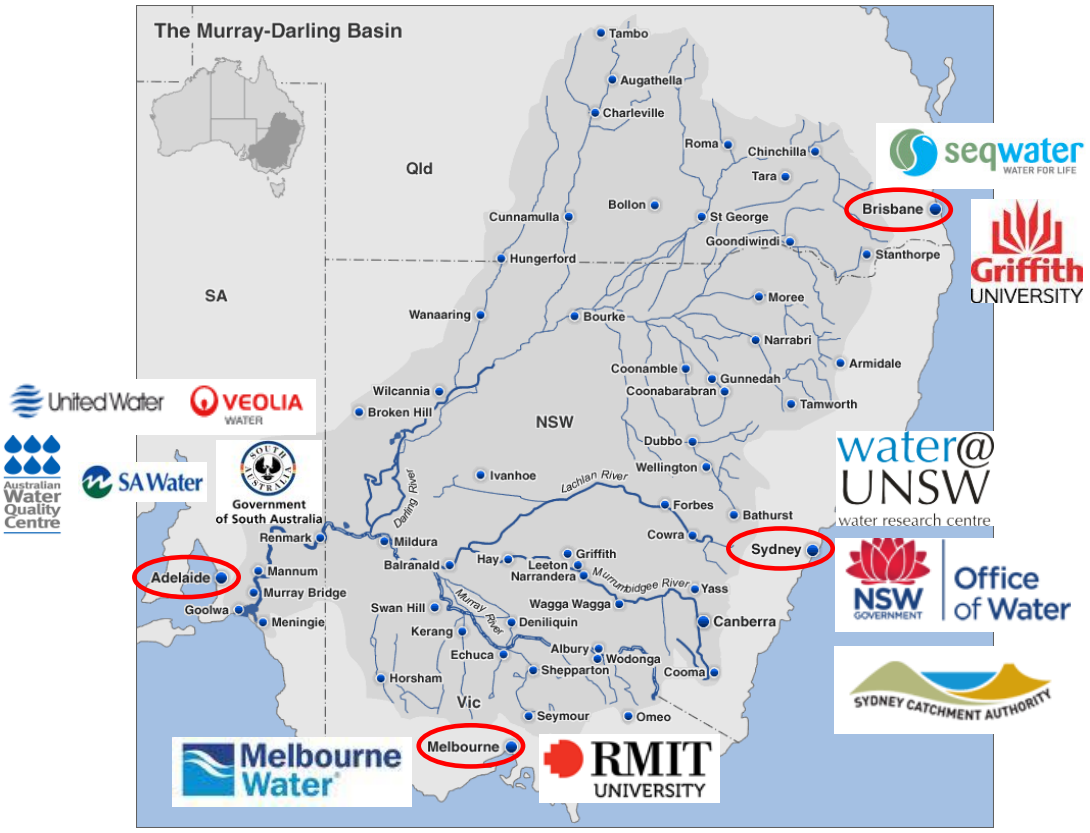

Figure 3.2: Map of the visited Australian water authorities and research centers over the Murray-Darling basin.

CHAPTER 4 PUBLICATION #1: CYANOBACTERIAL DETECTION USING *IN VIVO* FLUORESCENCE PROBES: MANAGING INTERFERENCES FOR IMPROVED DECISION-MAKING

This chapter presents the manuscript submitted to the JOURNAL OF AMERICAN WATER WORKS ASSOCIATION (JAWWA) 2011. This manuscript presents the sources of bias involved with application of *in vivo* PC probes for rapid CB detection, a possible correction factor and their applicability with regard to management threshold values in the field.

Cyanobacterial detection using *in vivo* fluorescence probes: managing interferences for improved decision-making

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4.1 Abstract

The applications of *in vivo* probes, which can detect the fluorescence of the cyanobacterial phycocyanin, are an emerging and widely used technology for cyanobacterial detection in source waters. The objectives of this project were to study the sources of interferences involved with readings of five probes using laboratory cultures and field samples. In order to compare the raw readings of different probes a novel approach was used to present the probe readings in biovolume equivalent of cyanobacteria. Inorganic turbidity and the presence of algal biomass were found to be two sources of interference involved with probe readings. A correction factor was developed for the probes with simultaneous chlorophyll *a* measurement. The field data demonstrate that the potential underestimation of status of the cyanobacterial population that corresponds to actual Alert Levels that are used to trigger monitoring and management actions is a major issue involved with application of *in vivo* probes.

4.2 Introduction

Global climate change may have profound effects upon watershed runoff and phosphorus loads thereby affecting the ecological state of water bodies. In addition, potential water shortages due to prolonged droughts, and elevated water temperature (Jeppesen, 2009), may enhance the dominance of potentially toxic cyanobacteria (CB) and their proliferation in drinking water (DW) sources (Dale, 2006; Elliott et al., 2006; Jöhnk et al., 2008; Paul, 2008). Infrequent sampling is conducted in most standard field monitoring programs (Chorus et al., 1999; Newcombe et al., 2010) and the recommended frequencies misses many pollutants loading events (AWWA, 2010; Zamyadi et al., 2007) that occur on smaller time scale including CB proliferation. Rapid and more frequent field monitoring of *in vivo* concentrations of CB is required for the effective management of the risks associated with the presence of toxic CB for recreational activities (Pilotto et al., 2008), DW production (McQuaid et al., 2011) and water reuse (McFarlane et al., 2008). Currently, the planktonic biomass is generally monitored by a combination of laboratory methods, including the measurement of chlorophyll *a* (Chl*a*), microscopic counting and taxonomic identification (AWWA, 2010; Bastien et al., 2011; Newcombe et al., 2010). Chl*a* has been used for many years as an indicator of phytoplanktonic biomass and is not specific to CB, as it is present in eukaryotic algae. Microscopic enumeration measurements provide information on the number of cells and their taxonomic distribution, but

may be subject to considerable systematic and operator error, particularly when filaments or colonies are present (Ahn et al., 2007; Lawton et al., 1999). In addition to being error-prone, they are tedious as several steps must be taken to prepare the samples requiring specialized equipment operated by experts (Gregor et al., 2007; Ziegmann et al., 2010). Other methods, such as continuous flow cytometry and high-resolution digital photometry, can be used for the task of counting and speciation of CB to the level of genus, and even of species (Daly et al., 2007). Real time quantitative polymerase chain reaction (qPCR) is also a promising technique (Fortin, 2010). However, these methods require skilled personnel, expensive instrumentations and they are not yet available as an "off the shelf" technology.

Tools to measure photosynthetic pigments based on fluorescence using light-emitting diode (LED) sources have been adapted to estimate the biomass of the main groups making up phytoplankton (chlorophyta, CB, brown algae – diatoms and dinoflagellates and the cryptophyta) by applying spectral signature differentiation algorithms (Beutler et al., 2002;Parésys et al., 2005; Wilhelm, 2003) . All these tools are based on the targeted excitation of accessory pigments that transfer photons to the Chl*a* terminal receptor, which then emits a particular wavelength (Krause et al., 1991). More in-depth analysis of the spectral signatures makes it possible to distinguish among groups of CB, e.g. blue and red (Beatler et al., 2004; Beutler et al., 2003). CB possess phycobiliprotein specific accessory pigments, phycocyanin (PC) and phycoerythrin (PE), and "off the shelf" probes for their detection are commercially available (AWWA, 2010; Gregor et al., 2007; McQuaid et al., 2011). PC has a characteristic fluorescence signature, which does not interfere with that of chlorophyll and makes it possible to distinguish the cyanobacterial biomass within the phytoplankton community (Fogg et al., 1973). PC absorbs red and orange light at wavelengths of 610-630 nm (maximum absorption at 620 nm) and emits fluorescence in the wavelengths band of 600-700 nm (maximum emission at 647 nm) (Carr et al., 1982; Fogg et al., 1973; Prescott et al., 2005).

This type of measurement has many advantages, including its specificity, relative simplicity of operation, fast response, portability (well adapted to the establishment of profiles covering a number of points in a source), and can be used online for intensive monitoring at a relatively modest cost (Asai et al., 2001; Izydorczyk et al., 2005). However, their use for management purposes raises a number of questions concerning their precision and their specificity in environmental conditions. These questions become particularly pertinent when using estimates

from this technology to trigger health advisories and intervention (McQuaid et al., 2011). If thresholds are defined by the numbers or the biomass of CB, the use of *in vivo* PC probes requires the use of conversion factors to express the relative fluorescence recorded by the probes into the unit used by the authorities. Threshold values may be expressed in number of CB cells, biovolume of CB and pigment concentration (e.g. World Health Organization thresholds for Alert Level 2 are 100,000 cells/mL, biovolume of 10 mm^3/L or 50 µg/L Chl*a*) (Chorus et al., 1999) and the relationship between these units are complex and site specific (Newcombe et al., 2010). Moreover, no guideline is available regarding the interpretation of data generated by this type of probe or regarding the need to measure other parameters (e.g. Chl*a* and water turbidity) simultaneously to improve their interpretation.

The response of *in vivo* fluorescence measurements is influenced by the selection of the excitation and emission spectra filters adjusted to the differences between the optimal excitation and emission wavelengths that defines the sensitivity and specificity of spectral resolution (Seppala et al., 2007). The spectra of the equipment available commercially are not necessarily optimal for all the species present (Beutler et al., 2003; Pemberton et al., 2007; Seppala et al., 2007). LED sources used for PC excitation are not always completely specific to PC and induce a certain degree of excitation of the Chl*a*. Indeed diatoms and chlorophytes interfered with *in vivo* PC signals by up to 10 µgPC/L when high densities of 100,000 cells/mL were measured using an *in vivo* PC probe (Brient et al., 2008). Similar inaccuracies occurred in other studies when *in vivo* fluorescence was employed to measure low cyanobacterial biovolume values in the presence of high Chl*a* concentrations as a result of high numbers of eukaryotic algae (Gregor et al., 2007; Izydorczyk et al., 2009). Simultaneous monitoring of eukaryotic algae fluorescence had been suggested to avoid false positive PC readings (Gregor et al., 2007).

Furthermore, environmental factors influence the quantity of Chl*a* and PC present in cells of a given species over time and unit cell content varies between species (Reynolds, 1984; Richardson et al., 2010; Seppala et al., 2007). Prior prolonged light exposure or bleaching may impact the intensity or signature of the PC (Donkor et al., 1996). However, *Planktothrix agardhii* exposure to 0-1900 µmol m^{-2} s^{-1} of natural light for 30 minutes has no influence on the *in vivo* detection of PC fluorescence (Brient et al., 2008). Abiotic factors such as turbidity can interfere with the transmission of the excitation wavelength and the cells‟ response to the probe. Information on the interference of turbidity is limited (Brient et al., 2008) but one manufacturer

recognizes and recommends a correction to the PC reading bias (21 cells-equivalent PC/NTU). Overall, *in vivo* fluorescence measurements require frequent validation with more robust methods for better speciation and biomass correlation (AWWA, 2010; Seppala et al., 2007).

The main objective of the research is to validate the use of *in vivo* PC probes for rapid CB assessment in surface waters. The specific objectives are: (1) to compare the performance of three commercially available *in vivo* PC probes, (2) to verify the importance of two sources of fluorescence interference, notably the simultaneous detection of Chl*a* emission from eukaryotic algae, and turbidity and to propose correction factors, (3) to study the applicability of these probes for management of drinking water (DW) sources, and (4) to monitor the variations in algal Chl*a* and PC as indicators to provide early warnings to plant operators.

4.3 Material and methods

4.3.1 In *vivo* fluorescence probes

In vivo fluorescence probes from three commercial manufacturers, probe A-PC[1], probe A-Chl*a*[2], probe B-PC[3] and probe C-PC[4], were used in this study (Table 4.1). Probe A-PC and probe A-Chl*a* and an optic turbidity probe[5] were installed on an online water quality multi-probe system[6]. These optical probes are equipped with a self cleaning system (wiper) installed in each probe. Prototype1[7] and Prototype2[7] *in vivo* PC probes and PrototypeChl*a*[7] *in vivo* Chl*a* probe were installed on a separate multi-probe system. Probe B-PC is the second *in vivo* PC probe used in this study which is also a submersible fluorometer. The probe C-PC, the third probe studied, is a submersible spectrofluorometer for algal *in vivo* detection. The LED of this probe emits six wavelengths to differentiate between different algae species based upon the different fluorescence excitation mechanism of different algae. However, in this study only the measurements with 610 nm excitation wavelength for CB detection were used.

In this study, only the raw readings of probes were recorded, so the manufacturers‟ original calibration was used for all probe readings. For probes A-PC and A-Chl*a*, the zero was adjusted using distilled water. The turbidity probe was calibrated using two points, the zero point with distilled water and a suspension of 124 NTU provided by the manufacturer. After calibration the entire multi-probe system was rinsed three times with distilled water.

Table 4.1: Specifications of *in vivo* fluorescence probes used in this study (YSI Inc., 2006; Moldaenke, 2009/08; Trios Optical Sensor, 2009; McQuaid et al, 2011; Bastien et al, 2011)

***In vivo* probe**	**Excitation wavelength (nm)**	**Emission wavelength (nm)**	**Resolution/ range**	**Detection limit**	**Limit of quantification**	**Raw probe reading unit**
Probe A-PC Prototype1 Prototype2 Probe A-Chl*a* Prototype-Chl*a*	590(bandpass: 15) 590 610 470 470	660 (bandpass: 20) 685 (bandpass: 40) 685 (bandpass: 40) 680 685	0.1/ 0-100 *	0.2*	0.7*	ratio fluorescence unit (RFU)
Probe B-PC	620	655 (bandpass: 5)	0.1/ 0-200	0.7 **	2.3 **	µg PC /l
Probe C-PC	610	680	0.05/ 0-200	0.3*	0.6*	µg Chl*a* /l

* Based on measurements performed on a monoculture of *Microcystis aeruginosa*.

** Based on measurements performed on a monoculture of 4,000 cells/mL of *M. Aeruginosa*

[1] YSI 606131 & [2] YSI 606025, Yellow Springs Instrument (YSI), Yellow Springs, Ohio, USA; [3] MicroFlu-blue, TriOS GmbH, Oldenburg, Germany; [4] FluroProbe, biological·biophysical·engineering (bbe) Moldaenke GmbH, Kronshagen, Germany; [5] YSI 606136 & [6] YSI 6600 V2-4 & [7] Prototype (No trade name yet), Yellow Springs Instrument (YSI), Yellow Springs, Ohio, US

4.3.2 CB and eukaryote cultures

A strain of *Microcystis aeruginosa* isolated from Mt. Bold Reservoir, South Australia, a strain of *Microcystis aeruginosa* isolated from a bloom in Canada, a strain of *Anabaena circinalis*, isolated from Chaffy Dam, New South Wales, Australia, a strain of *Scenedesmus* from Australia, and a strain of *Pseudokirchneriella subcapitata* from Canada were cultured separately in the laboratory in ASM-1 medium according to the method of Gorham et al. (Gorham et al., 1964). These strains remained unicellular in culture and did not form colonies. 20 L of distilled water was fortified with nutrients according to the ASM-1 preparation, sterilized at 120^{o}C, adjusted for pH and inoculated with an ASM grown culture in stationary phase (stock ASM-1 culture of *A. circinalis* was 1,120,000 cells/mL). Cultures were incubated at 26^{o}C under 6 hours rotating light-darkness flux at light intensity of 70 µmol s^{-1} m^{-2}. Samples for cyanobacterial enumeration by

microscopy were treated with Lugol`s iodine and then counted at 20× magnification using inverted microscopy methods described previously (CRCFE, 1999).

4.3.3 Microscopic count, biovolume estimation, cell integrity test and toxins analysis

Cell counts with species identification and biovolume calculations were conducted on a compound microscope in a Sedgewick-Rafter counting chamber after preservation in Lugol‟s iodine (CRCFE, 1999; Lund et al., 1958; Wetzel, 2000). The required volume of stationary phase culture to be spiked in water to achieve desired cell suspension was adjusted based on the cell number in the culture. The number of cells in suspension after inoculation was confirmed by microscopic enumeration. Water samples upon inoculation and after the experiments showed no sign of loss of cell integrity due to the direct addition of cells to water (data not shown). Fluorescein diacetate (FDA) – propidium iodide (PI) staining was chosen to verify the cell integrity (details in Hurst et al. (Hurst et al., 2007)) as it has been previously used for the determination of CB cell integrity (Zamyadi et al., 2010). Toxin measurements were conducted in triplicate for microcystins (MC) using an Abraxis MC ELISA Plate (an enzyme-linked immunosorbent assay – Abraxis LLC, Pennsylvania, USA) and results expressed in MC-LR equivalent.

4.3.4 Laboratory validation essay

Water was sourced from (1) the Adelaide DW distribution system in South Australia, and (2) the Montreal DW distribution system in Quebec (Canada) and stored 24 hours to eliminate chlorine. Experiments were performed in containers under conditions of natural day light (no artificial fluorescence involved) at 20 ±2^{o}C. Serial dilutions of *A. circinalis, M. aeruginosa*, *Scenedesmus* and *Pseudokirchneriella subcapitata* in stationary phase were conducted.

Six concentrations of *M. aeruginosa* were used 1,000 to 33,000 cells/mL (biovolume 0.096 to 3.199 mm^3/L). Turbidity and *in vivo* Chl*a* measurements of CB and *Scenedesmus* suspensions were completed using the probe A-Chl*a* and the optic turbidity probe. In total, five mono-suspensions of *A. circinalis* (1,900 to 281,000 cells/mL), four mono-suspensions of *M. aeruginosa* (11,200 to 302,000 cell/mL) and four mono-suspensions of *Scenedesmus* (900 to 159,000 cell/mL) were prepared, to which were added two concentrations of *A. circinalis* (10,000 and 100,000 cells/mL).

The effect of mineral turbidity and algal turbidity was studied using two mineral turbidity suspensions of kaolin and bentonite. Suspensions of 42 g/L kaolin and 35 g/L of bentonite, equivalent to 6,983 NTU and 6,475 NTU, respectively were prepared in tap water, kept in fully mixed solutions using a mechanical magnetic stirring for at least 15 minutes. For the experiment comparing the probe A-PC and probe B-PC with constant number of *M. aeruginosa* (70,000 cells/mL equivalents to 3.5 mm^3/L) a homogenous kaolin solution was removed from the high turbidity suspension using a 100 mL syringe. The high turbidity sample was added to the same *M. aeruginosa* suspension to achieve 4, 15, 30 and 65 NTU. The high turbidity sample was added to the three *M. aeruginosa* suspensions (0.4, 2.3 and 5.0 mm^3/L) and parallel turbidity and CB measurements were performed using probe A-PC and the optic turbidity probe.

To study the interference of Chl*a* concentrations from eukaryotic algae, ten mono-suspensions of *Scenedesmus* (with *in vivo* Chl*a* concentrations of 1.7 to 69.2 µg/L) and five mono-suspensions of *Pseudokirchneriella subcapitata* (with *in vivo* Chl*a* concentrations of 6.0 to 60.0 µg/L) were prepared (No CB involved). *In vivo* PC measurements were conducted on the mono-suspensions of *Scenedesmus* using probe A-PC and B-PC, and on the mono-suspensions of *P. subcapitata* using the Prototype1 and Prototype2 probes. To study the impact of typical Chl*a* concentrations on *in vivo* PC readings five mono-suspensions of *M. aeruginosa* cell numbers (28,000 to 82,000 cells/mL) were prepared. Probe A-PC, probe B-PC and probe C-PC were used to measure the PC from these CB species. Afterwards, *P. subcapitata* cells were added to all five mono-suspensions to obtain an *in vivo* Chl*a* concentration of 13 µg/L, and *in vivo* PC measurements were repeated. Using Prototype1 and Prototype2, five *Scenedesmus* mono-suspensions of 2,400 to 34,000 cells/mL were prepared. After *in vivo* PC measurements using the Prototype1 and Prototype2 probes, *M. aeruginosa* cells were added to the *Scenedesmus* mono-suspensions to attain 16,400 cells/mL (1.6 mm^3/L) and measurements were repeated. Probes measurements were also conducted in a *M. aeruginosa* mono-suspension of 16,400 cells/mL.

Mixing was achieved by magnetic stirrer to avoid air bubbles. PC raw readings, *in vivo* Chl*a* and turbidity measurement values from the memory of probe systems were discharged into Excel™, screened and basic statistical analyses were performed using Excel™ and Statistica9.1© StatSoft Inc. Each series of probe measurements were conducted in triplicate.

4.3.5 Site description

In vivo PC probe measurements were made within a period of three years (2007, 2008 & 2009) during summer and fall five water bodies located in south-east of province of Quebec, Canada. The five monitoring sites included four DW intakes located on (1) Missisquoi Bay (MB) on the Canadian side of the Champlain Lake, (2) Yamaska River (YR), (3) Lake St. Louis, (4) St. Lawrence River, sector of the island of Montreal, and Roxton Lake as a recreational water body which is located within the YR basin. Previous studies highlighted the problematic nature of CB blooms in BM and the water bodies located within the YR basin (McQuaid et al., 2011). The water intakes on Lake St. Louis and St. Laurence River were selected to study the applicability of the *in vivo* probes for monitoring in water intakes for two large DWTP.

4.3.6 Field monitoring

The *in vivo* PC monitoring using the probe A-PC was conducted at the water intake of the DWTP before any treatment. Parallel to *in vivo* probe measurements, water samples were taken in triplicate and treated by Lugol`s iodine for microscopic enumeration and for total toxin measurement. These samples were kept in closed ice packs during the transport to the laboratory and then stored frozen at -25^{o}C until analyzed. These samples were subjected to three sonication cycles before the analysis for total toxins using the ELISA plates.

4.4 Results and Discussion

4.4.1 *In vivo* PC probes laboratory validation in mono- and mixed-suspension of CB and eukaryotic algae

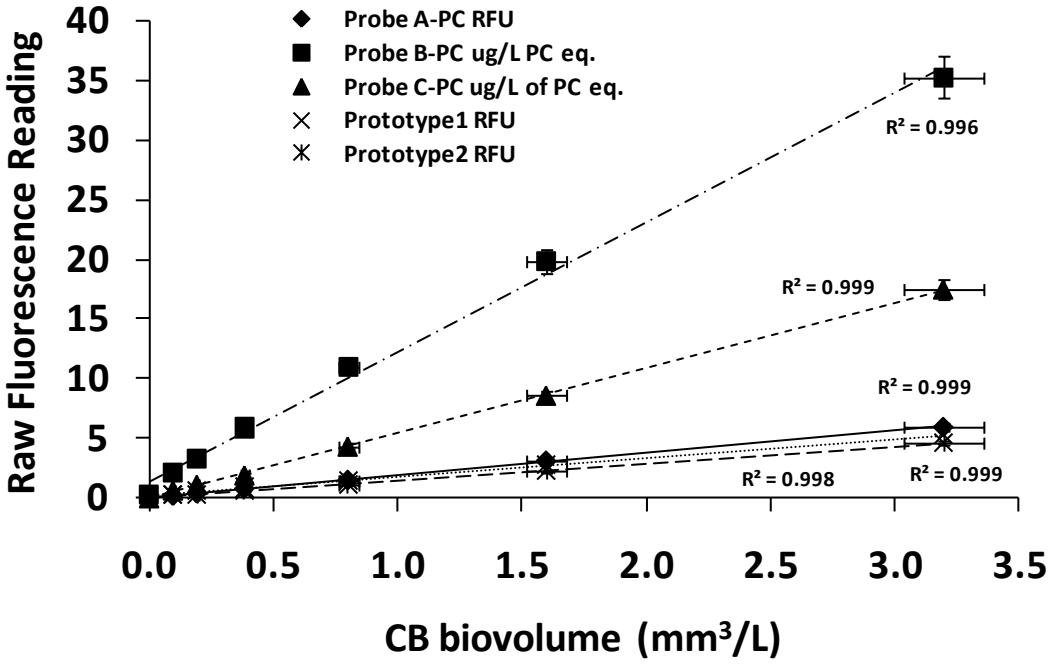

Figure 4.1: Relationship between the raw fluorescence output reading of the different *in vivo* PC probes and CB biovolume in six mono-cyanobacterial suspensions of *M. aeruginosa* (laboratory cultures with cells sourced from CB bloom in Canada and Australia, from low to high cell numbers, 1,000 to 33,000 cells/mL corresponding to biovolumes 0.1 to 3.2 mm3/L). Measurements were in triplicate and fitted regression lines and associate R2 vales are shown.

The relationships between the measured biovolume of *M. aeruginosa* estimated by microscopic enumeration and detected by PC probes using raw probes‟ reading units fitted to a linear regression show low variability for all probes tested (Figure 4.1). The repeatability of the probe measurements in low CB biovolume values was excellent (vertical standard deviation bars not visible) and remained very good at higher cell densities, while the variability of microscopic

enumeration increased with increasing cell numbers. These results are in accordance with those from Collins (2007) that showed a linear probe A-PC response over three orders of magnitude in varying cell concentrations (10^3 to 10^6 cells/mL). Using the probe B-PC, Brient et al. (2008), also demonstrated a linear probe signal response with high correlation coefficients ($R^2 \geq 0.994$) for low cell numbers of *Planktothrix agardhii* and *Lemmermanniella sp.*, as did Bastien et al. (2011) for probes A-PC and B-PC probes with *M. aeruginosa*. The *in vivo* measurement of the PC fluorescence by a probe similar to the probe C-PC allows the monitoring of cell densities under 150,000 CB cells/mL but could not be used above this threshold in Ribou lake, France (Cagnard et al., 2006).

Most probe systems transform raw probe readings into pigment equivalent concentrations (µgPC/L) or numbers of cell equivalent using *M. aeruginosa* as the benchmark CB. *M. aeruginosa* is indeed the dominant species in CB blooms in several regions (AFSSA-AFSSET, 2006) including Quebec (Canada) and microcystin-LR produced by *M. aeruginosa* is commonly found in source waters impacted by blooms (McQuaid et al., 2011; Ziegmann et al., 2010). The use of raw probe readings instead of the transformed results into cells equivalent/mL using the default manufacturers‟ conversion factor has been recommended, as raw fluorescence can be correlated to the total CB biovolume and the PC content (Bastien et al., 2011; Brient et al., 2008; McQuaid et al., 2011). The relative fluorescence corresponding to a number of cells is influenced by the CB species present, especially their varying interspecies biovolume (0.5-1022 mm^3/mL (Reynolds, 1984)) and their PC cell content (Reynolds, 1984; Richardson et al., 2010; Seppala et al., 2007;). Fluorescence can adequately predict biovolume for two species in a mono-species suspension (e.g. *Planktothrix agardhii* and *Lemmermanniella* sp.) but not cell numbers (Brient et al., 2008). It can be argued that a site specific conversion factor could be estimated using the biovolume of the dominant species identified by abundance classification, allowing the use of *in vivo* probe measurements for the direct estimation of cell numbers. However CB assemblages can vary in time and be composed of multiple species such as *Microcystis*, *Anabaena*, *Cylindrospermopsis*, *Aphanizomenon*, *Woronichinia* with wide ranges of biovolumes (McQuaid et al., 2011).

Expression of the raw fluorescence readings from different probes using a common metric is necessary to allow the true comparison of performance of these probes, or the estimation of the magnitude of interferences on their readings. For that purpose, a biovolume per unit of raw

fluorescence factor was calculated for each probe tested. Table 4.2 presents the resulting values of Fluorescence Biovolume Equivalent Unit (FBEU). These values were estimated from the average of the *in vivo* PC readings in six mono-suspensions of *M. aerugi*nosa using paired biovolumes and corresponding raw original readings of probes (Figure 4.1). For different biovolume values and raw probe readings, the FBEU ratio remains relatively constant.

Table 4.2: Values of Fluorescence Biovolume Equivalent Unit (FBEU - mm^3/L) for different probes corresponding to biovolume (mm^3/L) of *M. aeruginosa* in triplicate

PC *in vivo* Probe	A-PC	Prototype1	Prototype2	B-PC	C-PC
FBEU	0.50	0.50	0.70	0.07	0.18

The intrinsic contribution of CB and eukaryotic algae to probe readings of turbidity and Chla was verified before studying the bias of in vivo measurements. Figure 4.2a shows the turbidity associated with mono-cellular suspensions of CB and eukaryotic algae as measured by the optic turbidity probe. A direct linear relationship is observed between the number of CB and algae cells and measured turbidity, with a greater contribution per cell of A. circinalis because of its larger biovolume and its filamentous shape. Modest increases of in vivo concentration of Chla (<3.5ug/L) in mono-cyanobacterial suspensions are noted as a function of the number of CB enumerated by microscopy ($R^2 \geq 0.98$) (Figure 4.2b). Differences in the response between the two CB species probably reflect their reported Chl*a* cell content of 0.72 pg/cell for *A. circinalis* and 0.36 pg/cell for *M. aeruginosa* (Reynolds, 1984). As expected, *in vivo* readings are a significant underestimation of the Chl*a* pigment concentration per CB cell values because the major part of cyanobacterial Chl*a* is located in poorly-fluorescing photosystem I. Consequently, a specific feature for CB is a lower Chl*a in vivo* fluorescence when compared to that of eukaryotic algae (Campbell et al., 1998; Seppala et al., 2007). Figure 4.2c shows a clear linear relationship (R^2 = 0.93) between the number of *Scenedesmus* counted by microscopy and the concentration of *in vivo* Chl*a* (µg/L) in mono-cellular suspensions of eukaryotic algae (Figure 4.2c). These observations hold in two mixed suspensions of 10,000 and 100,000 cells/mL of *A. circinalis* and *Scenedesmus*. These results also show the overwhelming contribution of eukaryotic algae even in the presence of high concentrations of CB (Figure 2c). Environmental CB monitoring using *in*

vivo probes showed that Chl*a* and PC are generally weakly correlated (Ahn et al., 2007; Brient et al., 2008; Seppala et al., 2007).

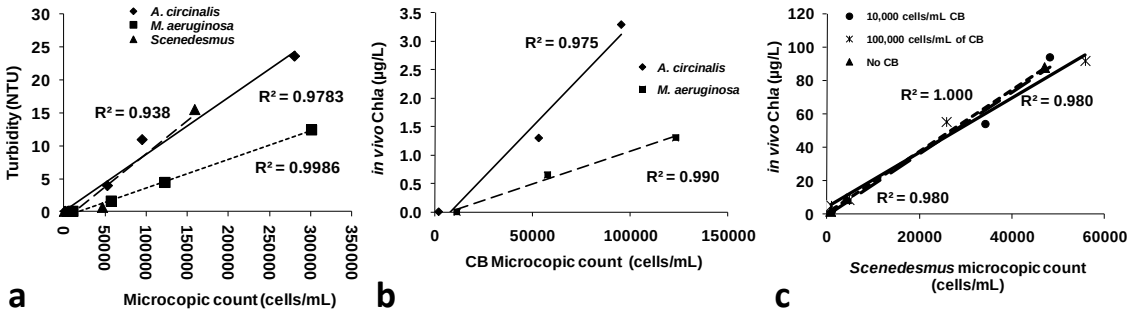

Figure 4.2: Turbidity and in vivo Chl*a* fluorescence associated with cell suspensions of two CB and a green algae as measured by sensor probes. (a) Turbidity vs. cell numbers over the range of 0-30,000 cells/mL; (b) *in vivo* Chl*a* readings for individual suspensions of *M. aeruginosa* and *A. circinalis*; (c) *in vivo* Chl*a* readings of mixed suspension of *Scenedesmus* with addition of two different concentrations of *A. circinalis* (10,000 and 100,000 cells/mL)

4.4.2 Bias involved with the probes readings and correction factor

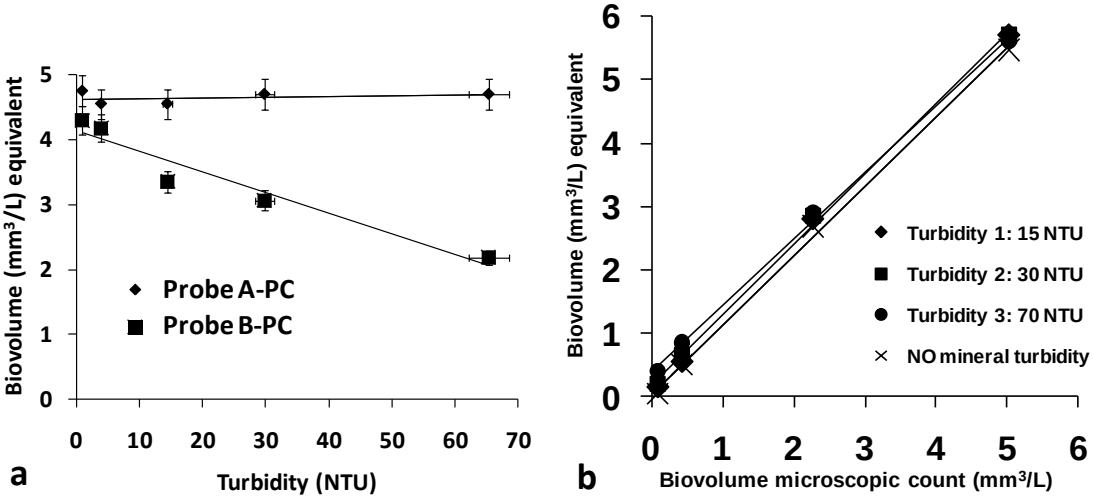

Figure 4.3: Interference of increasing concentration of mineral turbidity (a) kaolin and (b) bentonite on *M. aeruginosa* detection by Probe A-PC

The impact of turbidity by adding kaolin on the probe A-PC and probe B-PC was measured in a mono-suspension of *M. aeruginosa* (3.5 total biovolume mm^3/L and <1 NTU caused by CB cells). Raw readings of *M. aeruginosa* equivalent biovolume (mm^3/L) using the FBEU are plotted against the water turbidity values (Figure 4.3a). Repeatability of probe A-PC readings for triplicate tests was ±0.5 equivalent biovolume (mm^3/L) of *M. aeruginosa* as readings remained quasi constant with the increase in the water turbidity. In the case of probe B-PC, the presence of mineral turbidity in water samples decreased the signal of the probe leading to a maximum underestimation of 2.1 equivalent biovolume (mm^3/L) of *M. aeruginosa*, or approximately 48.8% of the biovolume present (Figure 4.3a). Turbidity has been shown to interfere with the probe B-PC, but the magnitude of the interference caused by sand (0.1g/l) depends on both the number and the size of the particles; small particles (0.1mm) reduced the fluorescence measurement by 12.3%, while larger particles (0.316μm) reduced it by only 5.9% (Brient et al., 2008). The relationship between microscopic enumeration results and the probe A-PC for different levels of turbidity (only CB and mineral mixed with CB) was assessed using a linear regression (all $R^2 = 0.99$ - Figure 4.3b) and results at different levels of turbidity clearly overlap (Figure 4.3b). Variability of the probe readings (for the same cell numbers) is not influenced by the turbidity and remains in the range of ±0.5 equivalent biovolume (mm^3/L) of *M. aeruginosa* of probe A-PC.

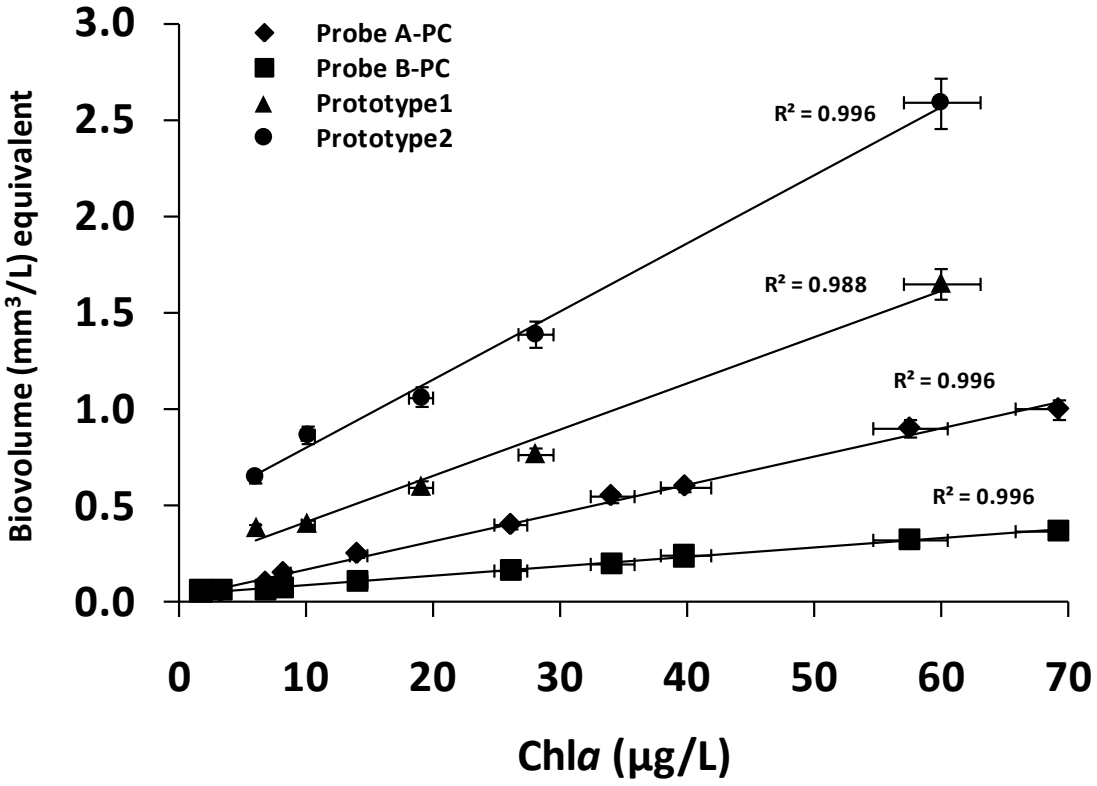

Figure 4.4: Contribution and potential interference of eukaryotic algae to *in vivo* fluorescence signal and CB measurement for the different probes. The effect of increasing concentrations of algae expressed as Chl*a* (in the absence of CB) to the *in vivo* PC readings given as CB Biovolume was measured for probe A-PC and probe B-PC with cultures of *Pseudokirchneriella subcapitata* and with *Scenedesmus* sp. for Prototype1 and Prototype2 probes.

Probe A-PC, Prototype1, Prototype2 and Probe B-PC readings in equivalent biovolume (mm^3/L) of *M. aeruginosa* are plotted against the increasing concentration of Chl*a* from *Pseudokirchneriella subcapitata* and *Scenedesmus* in absence of CB cells (Figure 4.4). Chl*a* concentrations used in this experiment are in the range of typical Chl*a* variations in Quebec (Canada) lakes (i.e. 1.4 to 60 µg/L) (Giani et al., 2005). False PC readings have been reported with probe A-PC and probe B-PC in the presence of high Chl*a* concentrations (Brient et al., 2008; McQuaid et al., 2011). Maximum values of probe A-PC and probe B-PC false readings reached 1.0 and 0.4 FBEU mm^3/L, respectively. Larger biases were observed with Prototype1 and Prototype2 probes suggesting that these prototypes were less selective for PC and would

require the use of a correction factor. Although the red fluorescence excited by wavelengths around 590±20 nm using a fluorescence reader is selective for CB, false positive signals may occur when a high amount of eukaryotic algae (e.g. diatoms) is present in the water (Gregor et al., 2007). The contribution of 100,000 cells/mL of the diatoms *Asterionella formosa* and chlorophyta *Scenedesmus opoliensis* to false PC measurement using the probe B-PC were reported as 6 and 3 µg/L-PC respectively (Brient et al., 2008).

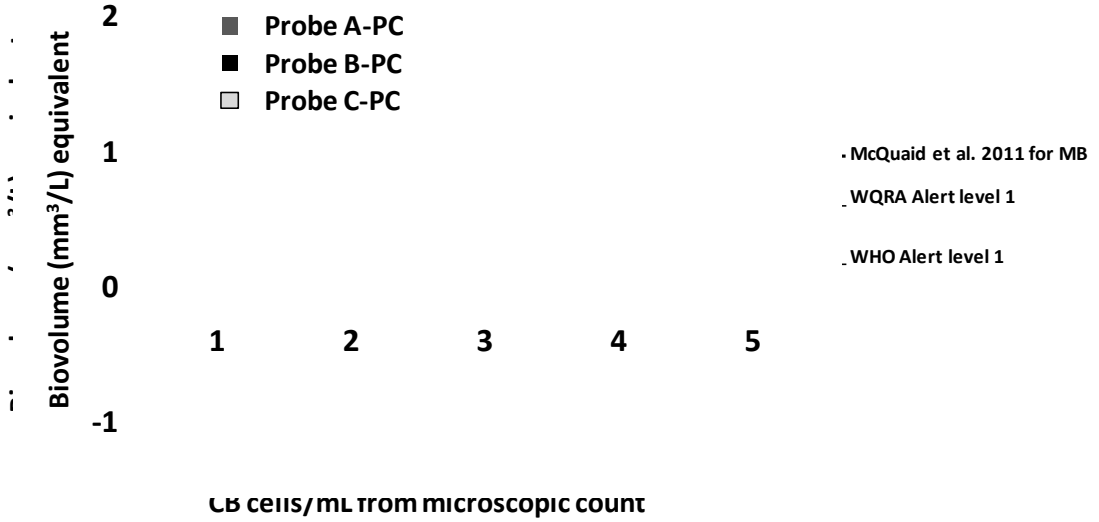

Figure 4.5: The difference between three *in vivo* PC probes‟ readings in mono-suspension of presence of *M. aeruginosa* and mixed suspension of *M. aeruginosa* and *Pseudokirchneriella subcapitata* (constant cell numbers with typical Chl*a* concentration of 13.0 µg/L).

The performances of probe A-PC, probe B-PC and probe C-PC to measure suspensions of *M. aeruginosa* cells in absence and presence of typical Chl*a* concentration (13.0 µg/L of Chl*a* caused by *Pseudokirchneriella subcapitata*) were compared. Figure 4.5 presents the differences between paired measurements with and without the addition of *P. subcapitata* for a range of CB concentrations from 28,000 to 82,000 cells/mL. The variability of the probes‟ response estimated from triplicate experiments did not increase (standard deviations ranging from ±0.05–0.14). No trend was noted with increasing concentrations. Overall differences with and without *P. subcapitata* cells were negligible for the probe B-PC (<0.001±0.05), intermediate for the bbe probe (0.07±0.28) and moderately higher for the probe A-PC (0.18±0.14). The significance of these differences in readings depends on the range of application, especially in reference to the

alert thresholds adopted for CB management plans and guidelines. As shown on Figure 4.5, the use of the PC probes tested in the presence of 13.0 μg/L of Chl*a* could lead to some false positive detections within the range of values of the lowest guideline proposed by WHO (Chorus et al., 1999). However, if a higher threshold is used, as the 0.6 mm^3/L set by WQRA (Newcombe et al., 2010) or the 1.0 mm^3/L proposed by McQuaid et al. (2011), the estimation errors for all tested probes appear manageable, provided that a verification of the eukaryotic algal biomass is performed in parallel with an *in vivo* Chl*a* reading.

The use of PC measurements to detect low densities of CB in the presence of high concentrations (>14 ug/L) of Chl*a* containing phytoplankton will likely lead to an overestimation of CB. However, from Figure 4.4, it can be seen that false measurements corresponding to the WQRA (Newcombe et al., 2010) and McQuaid et al. (2011) thresholds require considerable Chl*a* levels - 39 and 67 mm^3/L for the probe A-PC and 117 and 200 mm^3/L for the probe B-PC. The extent of this interference will depend on a number of factors, the most important being the concentration of Chl*a* present in the algal biomass which depends on the composition and state of the phytoplankton (Figure 4.4). In highly eutrophic lakes, the cyanobacterial biomass represents an increasing proportion (1-100%) of the total phytoplanktonic biomass (AWWA, 2010).

The precision of the emission signal and of its reading is determined by the technical specifications and operational state of the optics of the instruments. The extent of the Chl*a* interference thus depends directly on the selectivity of the optical components of the probe. Two strategies have been adopted by manufacturers to account for this interference: (1) a direct measurement of PC by selecting the most selective excitation and emission filters as done in probe A-PC, (2) measuring Chl*a* and other fluorescence signatures and using an algorithm to estimate the residual signature of PC as done in probe C-PC. When designing a low cost portable device, the trade off between specificity and cost may lead to accepting some Chl*a* interference in field samples, at least within a defined range of Chl*a* concentrations.

4.4.3 Development of the new generation of probes for *in vivo* CB monitoring

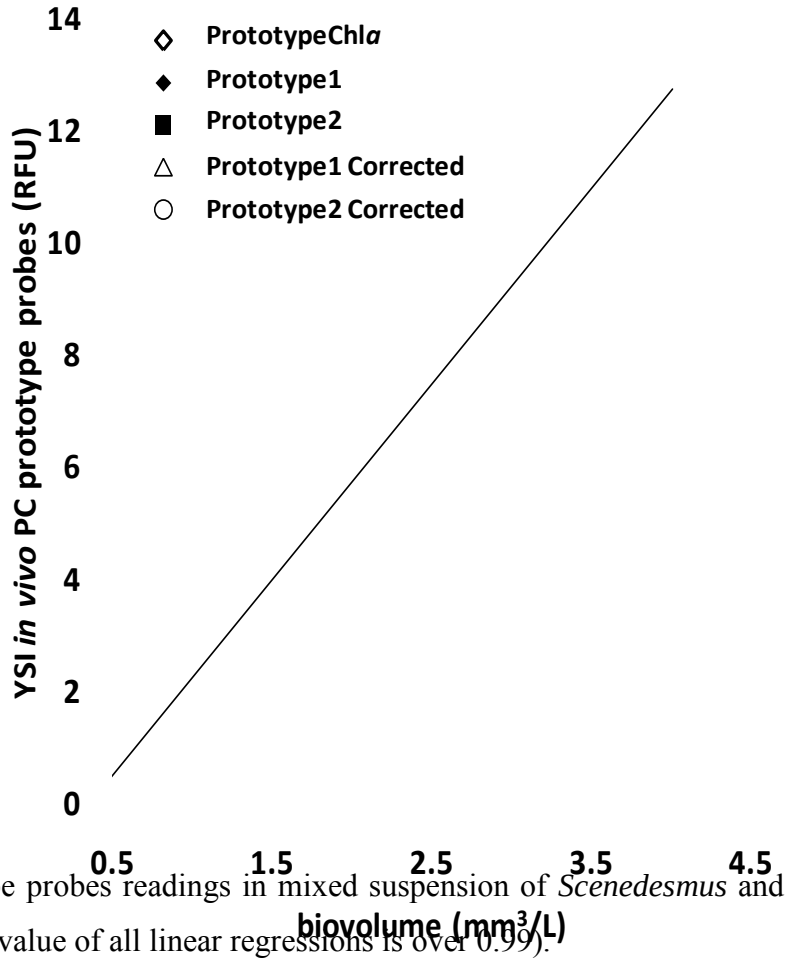

Figure 4.6: Prototype probes readings in mixed suspension of *Scenedesmus* and *M. aeruginosa* after correction (R2 value of all linear regressions is over 0.99).

An example of the success of this second strategy is shown with the Prototype1 and Prototype2 *in vivo* PC probes‟ readings in mono-suspensions of *Scenedesmus* and mixed suspensions of *Scenedesmus* and fixed concentrations of *M. aeruginosa* (Figure 4.6). The PC LED and photo detectors of these prototype probes are not designed to be more specific than the probe A-PC commercially available yet a reading of Chl*a* is taken simultaneously for correction purposes. PC probes excite and receive Chl*a* fluorescence signals corresponding to the false readings as exemplified Figure 4.4. The corrected PC reading can then be generated considering both the *in vivo* fluorescence of CB and the interference from Chl*a* from eukaryotic organisms to produce a Corrected *In vivo* PC Reading (CIPCR) using Equation 1.

$$CIPCR = (A \times invivoPC) \pm (B \times invivoChla) \qquad \text{Equation 1}$$

where *invivo*PC is the raw *in vivo* PC reading and *invivo*Chl*a* is the raw *in vivo* Chl*a* reading. Solving this equation is possible using paired probe readings in a mono suspension with a constant concentration of *M. aeruginosa* and no *Scenedesmus*, and a mixed suspension of *Scenedesmus* and *M. aeruginosa* (Figure 4.6). As the *M. aeruginosa* cell numbers were the same in both suspensions the CIPCR was set constant. The mono suspension of *M. aeruginosa* of 16,400 cell/mL yielded baseline estimates of the Prototype1 (1.358±0.006 RFU) and Prototype2 (1.104±0.003 RFU) probes. The application of this algorithm to a mixed suspension of *Scenedesmus* and *M. aeruginosa* provided highly satisfactory corrections with values of 1.357±0.007 RFU for Prototype1 and 1.105±0.002 RFU for Prototype2 (Figure 4.6). Notably, in Figure 4.6, the resulting PC readings were strongly similar in the presence and absence of *Scenedesmus*.

4.4.4 Field validation of the *in vivo* readings and application of the alert levels

Taxonomic microscopic CB enumerations were completed in samples collected over a period of three years in five sources in Quebec (Canada): Missisquoi Bay (MB), Yamaska River (YR), Lake Roxton, the Atwater Canal and Lake St-Louis. Microscopic taxonomic identification of CB in samples from MB and YR revealed that, on average, 83% of the CB biovolume was composed of potentially MC producing species with *Microcystis* sp. (*M. flos-aquae*, *M. aeruginosa* and *M. wesenbergii*) being the dominant genus. *Anabaena flos-aquae*, *Anabaena spiroides crassa*, *Planktothrix* sp., *Oscillatoria tenuis* and *Woronichinia naegiliana* were also present. In samples from Lake Roxton the dominant genus was *Aphanizomenon* sp. (*A. flos-aquae* and *A. flexuosum*) with some *Woronichinia naegiliana* and *Anabaena* sp. In the Atwater source, the dominant species was *Aphanothece clathrata brevis* while *Microcystis* sp., *Coelosphaerium kuetzingianum* and *Oscillatoria limnetica* were also detected. *Aphanothece clathrata brevis* was the dominant species in samples from Lake St. Louis with some presence of *Coelosphaerium kuetzingianum* and benthic filamentous *Lyngbya* sp.

The application of the probe will be judged primarily on its ability to correctly detect significant events above the threshold limits, and, to a lesser extent minimizing false positives. Figure 4.7 shows the correlation between *in vivo* PC measurements and microscopic biomass estimates of indigenous CBs from five surface water sites. Samples with cell counts exceeding 200,000 cells/mL were not considered for validation because of probe detection limits, but it may be

noted that concentrations of cyanotoxins measured in these samples ranged from 1.2 to 14 µg/l of MC-LR equivalents.

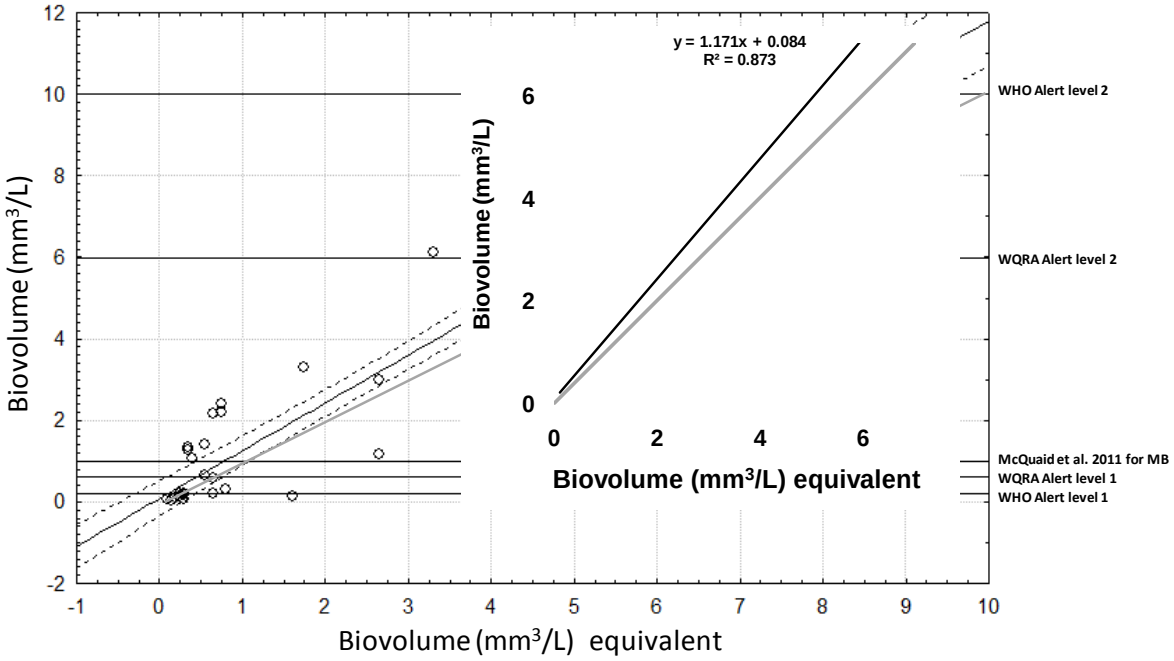

Figure 4.7: Probe PC-A readings in biovolume equivalent of *M. aeruginosa* VS biovolume from microscopic count in field samples, with the confidence limit of 95% and different alert levels.

Probe A-PC readings in biovolume (mm^3/L) equivalent (using FBEU) and their equivalent number of cells of *M. aeruginosa* are plotted against cell biovolumes from taxonomic microscopic estimations. Similar to laboratory results, a linear correlation is observed between biomass estimates derived from PC probes and cell biovolumes. In our case, using the FBEU, we can correlate the measured biomass using the two methods. The line of equivalence corresponds to an equivalent estimation by the *in vivo* and microscopic estimations. The correlation obtained in Figure 4.7 is drawn from 46 samples from 5 locations shows a strong correlation coefficient ($R^2 = 0.87$, $p < 0.01$), a coherent intercept and relatively narrow 95% confidence intervals, which are important aspects to consider for field application. These results of environmental samples from multiple sites compare advantageously to previous reports. Bastien et al. (2011) correlated probe A-PC readings (default transformation of RFU to Log cells/mL) against biovolumes (Log mm^3/L) over a very wide range of biovolumes 1-6,000mm^3/L using 91 field samples from 20 sites ($R^2 = 0.73$) with a maximum dispersion of ~10 (1 log) mm^3/L. A slightly better correlation

(R^2 = 0.73) with similar scattering was observed using a probe B-PC (Bastien et al., 2011). However, dilution was used to generate several higher readings in the range of concentrations that exceeded the design capacity of the probes (*pers. comm.*). In a range of biovolumes directly applicable to the probes, Izydorczyk et al. (2009) correlated CB Chl*a* measured by a probe similar to the probe C-PC against CB biovolume with R^2 of 0.46 and maximum dispersion of 18 mm^3/L in 40 samples from one lake. Izydorczyk et al. (2009) concluded that the probe similar to the probe C-PC was an appropriate monitoring probe for the detection of toxic CB blooms at DW intakes.

Our data suggests that main sources of error include interferences to the *in vivo* readings (turbidity and Chl*a*), biases associated with the factors used to express fluorescence raw readings into biomass, varying PC cell content, and microscopic enumeration and biovolume estimation. The heterogeneity of CB cell presence in field samples (McQuaid et al., 2011) may explain the higher dispersion of probe data when transferred into cells/mL. Errors associated with microscopic cell counts can be significant, varying from 2-20% and increase for biovolume estimates (Newcombe et al., 2010). It is recognized that the cellular content of phycobiliprotein per cell of CB varies due to growth conditions and can be minimal in picocyanobacteria (Seppala et al., 2005; Seppala et al., 2007). PC *in vivo* fluorescence and biovolume of filamentous CB in the Baltic Sea were clearly correlated, but the variability in this correlation between transects was attributed to the variation in PC cell content cells (Seppala et al., 2007). Furthermore, Seppala et al. (2007) reported lower PC fluorescence in field results than that estimated in the laboratory for *Aphanizomenon sp.* and *Nodularia spumigena*. It is also possible that PC cell content is maximized under constant moderate light intensities applied in the culture room. Indeed, Figure 4.7 suggests that, overall, probe A-PC slightly underestimate the CB biomass estimated by microscopy by about 20% with some scatter (R^2 = 0.87). The main source of this underestimation may be the use of FBEU to translate raw RFU readings to biovolume, as it is based on measurements of cultured *M. aeruginosa*, while field samples are composed of varying CB species exposed to natural light conditions.

In light of the observed sources of error and interference, the most important factor to consider for the use of the tested *in vivo* probes is their ability to provide reliable data at threshold values used for management purposes. Completing the existing WHO alert levels (Alert Level 1 of ≥0.2 mm^3/L and <10 mm^3/L, Alert Level 2 ≥10 mm^3/L) (Chorus et al., 1999), Australian guidelines

have recently introduced three alert levels for CB monitoring in DW reservoirs, estimated using the toxic production potential of CB species: Alert Level 1 of $\geq$0.2 mm^3/L and <0.6 mm^3/L, Alert Level 2 $\geq$0.6 mm^3/L and <6 mm^3/L, and Alert Level 3 $\geq$6 mm^3/L (Newcombe et al., 2010). Also, McQuaid et al. (2011) proposed a CB biovolume based threshold of 1 mm^3/L for monitoring based on 3 years of field sampling simultaneously using the probe A-PC, microscopic enumeration and toxin analysis. Some of these threshold values are plotted on Figure 4.7. Our results suggest that the use of the probes is possible when considering intermediate and high alert levels of alert (Figure 4.7). In six data points out of 46, the use of the probe A-PC:biovolume trend line may lead, in some cases, to an underestimation of alert level (i.e. false negatives). Similar occurrence of false negatives and false positives can be observed in previously published data (Bastien et al., 2011; Izydorczyk et al., 2009). Izydorczyk et al. (2009) recommended the use of *in vivo* measurement of Chl*a* defined threshold levels of CBs in a DW intake corresponding to potential concentrations of MC-LR, but stress that these threshold levels are site specific. In other cases, the CB presence in water samples from Lake Ontario (North America, Canada) were generally underestimated in fluorescence measurements using probe C-PC (Pemberton et al., 2007). Richardson et al. (2010) observed a statistical difference between the measured abundance of phytoplankton using the *in vivo* probe similar to the probe C-PC and a more robust laboratory method. They reported that the total phytoplankton biomass was overestimated by a factor of 1.2 to 3.4 using the probe similar to the probe C-PC (Richardson et al., 2010). However, they conclude that the bbe probe was reasonably acceptable to monitor trends.

These observations all suggest that site specific calibration may improve the accuracy of the probe readings and their application to alert thresholds. Frequent calibration of the probe by discrete sample collection and species representative of the monitoring site was highly recommended for the *in vivo* probes (AWWA, 2010). It has been suggested that the confusion between different phycobiliprotein containing phytoplankton and variation in species present in different locations can be the source of these contradictory results for *in vivo* fluorescence measurements (Pemberton et al., 2007). Finally, remaining sources of false readings remain to be quantified. Our field observations identified the presence of phycobiliprotein containing cryptophytes. Cryptophytes are important primary producers in freshwater but their cells are delicate and rupture when fixatives are added to samples and when temperatures are elevated

(AWWA, 2010). They are prominent in oligotrophic waters (AWWA, 2010) and they contain PE, the phycobilisomes consisting of phycobiliproteins (Beutler et al., 2004).

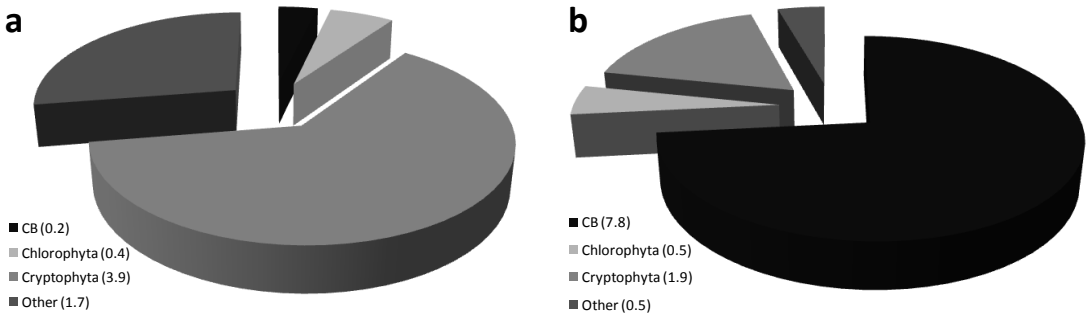

Figure 4.8: Proportion of biovolume of PC and Chla containing CB and different algae species out of total sample biovolume (a) MB and (b) Lake Roxton (the values in the parenthesis are the biovolume of different species in mm^3/L)

Figure 4.8a summarizes the relative abundance and total biomass of algae and CB in the MB before a CB bloom formation showing a clear dominance of cryptophytes (3.9 mm^3/L). Figure 4.8b presents the proportion of other algae species present during CB bloom event in Lake Roxton, showing also a significant presence of cryptophytes (1.9mm^3/L). These data are coherent with the observations of McQuaid et al. (2011) where the presence of chlorophyta and cryptophyta were detected in parallel to CB blooms. The importance of the false positive *in vivo* readings resulting from the presence of non-negligible biomass of cryptophyta cannot be assessed as the phycobiliprotein content of the species present in these samples is not known.

4.5 Conclusion

The laboratory validation of PC *in vivo* probes showed that their responses to the CB concentrations in mono and mixed suspensions were linear and sensitive. Controlled validation testing also showed significant interferences to *in vivo* fluorescence readings due to turbidity and fluorescence emissions of Chl*a* from eukaryotic algae. The Chl*a* interference in the presence of concentrations of Chl*a* (<14µg/L) is minor and can be accounted for. Probes with more selective optics or incorporating a correction factor based on dual PC-Chl*a* readings should be used if

higher concentrations of Chl*a* are present. The relative importance of these interferences varies between commercially available probes and should be taken into account when interpreting the online data.

PC *in vivo* probe readings were validated with microscopic taxonomic biomass estimates on field samples from five surface water sources. Significant sources of variations identified include inaccuracies in interspecies and intra species microscopic counts and biovolume estimation especially in highly diverse samples and the presence of Chl*a* containing chlorophyta and phycobiliprotein containing cryptophyta. Underestimation of alert levels using *in vivo* measurements is a critical issue for the application of probes in raw DW sources. *In vivo* fluorescence based detection of CB and microscopy-based identification and quantification of species support each other by providing complimentary information at different scales.

4.6 Acknowledgments

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CHAPTER 5 PUBLICATION #2: RELEASE AND OXIDATION OF CELL-BOUND SAXITOXINS DURING CHLORINATION OF ANABAENA CIRCINALIS CELLS

This chapter presents the paper published in the journal of ENVIRONMENTAL SCIENCE AND TECHNOLOGY 2010, VOL. 44, NO. 23, 9055–9061. This paper titled "Release and oxidation of cell-bound saxitoxins during chlorination of Anabaena circinalis cells" demonstrates that chlorination of *Anabaena circinalis* causes complete cell lysis, cell-bound geosmin and saxitoxins release, and over 95% toxin oxidation within the disinfection by-products guidelines.

Release and oxidation of cell-bound saxitoxins during chlorination of *Anabaena circinalis* cells

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5.1 Abstract

Surface water sources are increasingly subject to proliferation of toxic cyanobacteria. Direct chlorination of source water containing toxic cyanobacterial cells for different treatment purposes might cause cell damage and toxin release. There is limited information available on chlorination of saxitoxins (STXs: saxitoxin, C-toxins and, gonyautoxins) produced by *Anabaena circinalis*. This study: (1) investigated the impact of chlorination on cell lysis and toxin/odor compound release in natural waters; (2) assessed the rates of chlorination of total STXs, and (3) estimated apparent rate constants for STXs oxidation in ultrapure and natural waters. With chlorine exposure (CT) value of 7.0 mg.min/L all cells lost viability causing toxin release. Cell membranes damage occurred faster than released STXs oxidation. All saxitoxin and more than 95% of other STXs analogues were subsequently oxidized. Kinetic analysis of the oxidation of STXs analogues revealed significant differences in the susceptibility to chlorine, saxitoxin being the easiest to oxidize. Also, concentrations of trihalomethanes, haloacetic acids, and *N*-nitrosodimethylamine as chlorination by-products were respectively <50 µg/L and 11 ng/L even at the highest CT value (50.3 mg.min/L).

5.2 Introduction

Many potentially toxic cyanobacteria (CB) species and their associated toxins have been increasingly detected in surface source water and treated drinking water (DW) (AFSSA-AFSSET, 2006; Lahti et al., 2001). Saxitoxins (STXs) also known as paralytic shellfish poisons are one of the main toxins of interest (Duy et al., 2000). STXs are alkaloids based on a 3,4,6-trialkyl tetrahydropurine skeleton which can be further carbamylated, sulphated or N-sulphocarbamylated to produce a range of 30 analogues, some of which are found only in freshwater cyanobacteria (Humpage, 2008; Nicholson et al., 2003). The more commonly found analogues are saxitoxin (STX), C-toxin1 (C1), C-toxin2 (C2), gonyautoxin2 (GTX2) and gonyautoxin3 (GTX3). *Anabaena circinalis* is one the main CB species producing these neurotoxins (AFSSA-AFSSET, 2006; Nicholson et al., 2003). Freshwater *A. circinalis* blooms have been found worldwide (Merel et al., 2010). STXs are responsible for the discontinuous but repeated widespread poisoning of wild and domestic animals, fish and more recently, humans (Merel et al., 2010; Svrcek & Smith, 2004). *Anabaena* sp. are also responsible for many odour

episodes in water sources around the world. Geosmin (trans-1,10-dimethyl-trans-9-decalol) is one of the earthy-odour compounds that can be produced by *Anabaena* sp. (Lin et al., 2009).

Based on acute toxicity tests the dosages of STXs lethal to 50% of the test population (LD_{50}) range from 10 to 30 (mouse μg/kg body weight) making them one of the most hazardous neurotoxins (Codd et al., 2005). Most neurotoxins are acute-acting, that is, they act immediately at very low doses. STXs are fast-acting neurotoxins that are potent voltage-gated sodium channel antagonists which cause numbness, paralysis and death by respiratory arrest (Humpage, 2008). STX disrupts the nervous system via binding to the sodium channel and inhibits the sodium ions transport (Catterall, 1980). Consequently, a health-based exposure guideline level of 3.0 μg/L has been proposed for STX toxicity equivalent in Australia, New Zealand and Brazil (Australia, National Health and Medical Research Council and Natural Resource Management Ministerial Council, 2004; Molica et al., 2005; Ministry of Health New Zealand,2005). The relative toxicity of other STXs analogues varies widely but is lower than that of STX (Jellett et al., 1995).

It is anticipated that climate change will increase the frequency and intensity of cyanobacterial blooms thus impacting DW intakes (Elliott et al., 2006; Johnk et al., 2008). Direct chlorination of raw water is used for several water treatment purposes (e.g. unfiltered DW production and filter cycle optimization) (Chen et al., 2005; Petrusevski et al., 1995). Direct chlorination of source water containing toxic CB cells raises several water quality concerns such as: (1) cell damage and the subsequent release of cell-bound toxins (Daly et al., 2007); (2) increased chlorine demand (Daly et al., 2007; El-Did & Ali, 1994), (3) dissolved organic carbon (DOC) release (Daly et al., 2007; Peterson et al., 1995), (4) geosmin release (Lin et al., 2009; Peterson et al., 1995), and (5) elevated chlorination by-products, e.g. trihalomethanes (THM), haloacetic acids (HAA) and/or N-nitrosodimethylamine (NDMA) (Hoehn et al., 1980; Merel et al., 2010). Consequently, there is a need to understand the limitations of the oxidation of CB cells and their toxins. Pre-chlorination doses can vary from 0.25 to 2 mg/L and are adjusted according to ammonia concentrations and not the concentration of CB and/or cyanotoxins (AFSSA-AFSSET, 2006). Results from limited information on the impact of pre-chlorination doses on oxidation of cell-bound toxins are contradictory (Carlile, 1994; Nicholson et al., 1994).

Chlorine has the potential to effectively oxidize *A. circinalis* cells and STXs under specific conditions (Ho et al., 2009; Ho et al., 2006; Nicholson et al., 2003). Overall STXs removal by

chlorine was more effective at higher pH values (>8) compared with lower pH values (5 and 7) (Nicholson et al., 2003; Cooperative Research Centre (CRC) for Water Quality and Treatment, 2005). However, chlorination at elevated pH is not optimal for removal of microcystin (MC) toxins and cylindrospermopsin (CYN) (Newcombe & Nicholson, 2004). It was concluded that oxidation techniques do not appear to be the best method for the treatment of STXs under normal treatment plant operating conditions (Newcombe & Nicholson, 2004). However, recent observations of STX chlorination in natural water suggest that the pH dependency in the range of 7 to 8 may not be as important as reported earlier (Nicholson et al., 2003; Cooperative Research Centre (CRC) for Water Quality and Treatment, 2005).

Currently, apparent rate constants for the reaction of STXs with different oxidants are unknown. Furthermore, chlorination is largely ineffective for geosmin oxidation (Ho et al., 2009). For the confident application of chlorine for oxidation purposes the important parameters are the concentration of chlorine and the time over which the cells and/or toxin are exposed. The appropriate degree of oxidation is obtained using the concept of concentration multiplied by time, chlorine exposure, or CT. The CT value is calculated by determining the area under a graph of chlorine concentration vs. time (Ho et al., 2006). Total (dissolved and cell-bound) toxin oxidation can be predicted by quantifying the rates of cell lysis, toxin release, and toxin oxidation under given water quality conditions (Daly et al., 2007; Schmidt et al., 2002).

Systematic studies are needed to better understand the STXs producing CB cells oxidation by chlorine and subsequent toxin release and oxidation (Merel et al., 2010). The objectives of this research were: (1) to determine the extent of *A. circinalis* cell lysis and STXs/geosmin release in natural water; (2) to assess the rates of oxidation of STXs by free chlorine; (3) to estimate apparent rate constants for STXs oxidation in ultrapure and natural waters. To the best of our knowledge, this paper presents the first estimations of apparent rate constants of STXs oxidation by chlorine.

5.3 Experimental Procedures

Materials and Reagents. A toxic strain of *A. circinalis*, isolated from Chaffy Dam, New South Wales, Australia, was cultured in the laboratory in artificial seawater medium (ASM-1) according to the method of Gorham et al. (Gorham et al., 1964). Distilled water (20 L) was fortified with nutrients according to the ASM-1 preparation, sterilized at 120^{o}C, and then after

pH adjustment, inoculated with an ASM-1 grown culture in stationary phase (stock ASM-1 culture of *A. circinalis* was 1,060,000 cells/mL). The culture was incubated at 26^{o}C under 6 hours rotating light-darkness flux at light intensity of 70 µmol s^{-1} m^{-2}.

Toxin spiking experiments were conducted using STXs extracted from a local natural bloom of *A. circinalis* that occurred at Myponga Reservoir in South Australia in late October of 2006. The isolation procedure involved freeze-thawing the bloom material in 75% methanol. The toxin retentate was purified and passed through ultra-filtration. The final concentrated extract also used by Ho et al. (Ho et al., 2009) contained C1 (97 mg/L), C2 (98 mg/L), GTX2 (6.7 mg/L), GTX3 (6.3 mg/L), and STX (3.5 mg/L). Certified reference material for STXs was purchased from the National Research Council of Canada and used as standards for qualification/quantification purposes. All chemicals and reagents were laboratory analytical grade from various suppliers.

Water Quality Characterization. Water was sourced from the Murray River (MR) in South Australia. Samples were collected from the Morgan water treatment plant intake prior to any treatment. Untreated MR water was filtered over pre-rinsed Binder-free glass microfiber 1.2µm filter (GF/C - Whatman, England) prior to inoculation with culture. The water had a DOC concentration of 2.4 mg/L, UV absorbance (at 254 nm) of 0.045 cm^{-1}, pH of 6.8, and an alkalinity of 88 mg/L as $CaCO_3$. Ultrapure water (Millipore Pty Ltd, USA) was also used for selected experiments. Prior to DOC analyses, samples were passed through pre-rinsed 0.45 µm cellulose nitrate membrane filters (Schleicher and Schuell, Germany). DOC measurements were made on an 820 total organic carbon analyzer (Sievers Instruments Inc., USA). pH was measured on a PHI 50 pH meter (Beckman Instruments, USA) which was calibrated with pH 4, 7 and 10 standard buffers (BDH, Australia).

Chlorination Experiments (Cl_2 Exp). A chlorine stock solution was prepared by bubbling gaseous chlorine through ultrapure water in a glass flask. The flask was then sealed and stored at 4^{o}C for at least 16 hours prior to use (Ho et al., 2006). Chlorine stock and chlorine residual concentrations were determined using the DPD-FAS titration method described in Standard Methods (American Public Health Association, 1998). Typical chlorine stock solutions concentrations ranged from 2000-6000 mg/L as free chlorine. Two separate chlorination experiments were conducted on (1st Cl_2 Exp) STXs producing *A. circinalis* suspensions and (2nd Cl_2 Exp) STXs extracts dilutions. Each chlorination experiment was presided by a chlorine decay

experiment. For chlorine decay experiments, water samples were dosed with desired chlorine concentration and triplicate samples taken at specified contact times for free chlorine residual determinations using the titration method. All chlorination experiments were conducted in triplicate in brown amber bottles at room temperature ($20 \pm 2^{o}C$).

1st Cl_2 Exp: Cultured *A. circinalis* cells were enumerated on a compound microscope in a Sedgewick-Rafter counting chamber after preservation in Lugol‟s iodine (Cooperative Research Centre for Freshwater Ecology, 1999). Required volume of stationary phase culture to be spiked in MR water to achieve desired cell suspension was adjusted based on the cell number in the culture. The number of cells in MR water after inoculation was confirmed by microscopic enumeration. Control (non-chlorinated) water samples showed no sign of cell integrity loss due to direct addition of cells to MR water (data not shown). The pH of water samples were maintained at 8 by the addition of phosphate buffer (0.01 M) (American Public Health Association et al., 1998). Chlorine was added from the chlorine stock solution to obtain the desired doses (2 and 3 mg/L). Samples were quenched at specified contact times with sodium thiosulphate at a stoichiometric ratio specified in Standard Methods (American Public Health Association et al., 1998). Quenched samples were taken for DOC measurement. To determine cell viability two microscopic counts were conducted in triplicate, (1) cell integrity tests maximum 30 minutes after the end of experiment on non-preserved 2 mL samples, and (2) total cell counts on 2 mL samples after preservation in Lugol‟s iodine (Cooperative Research Centre for Freshwater Ecology, 1999). Remaining quenched water samples were divided in two subsamples of 250 mL: the first subsample was immediately filtered through 0.45 μm filter (Whatman, UK) to remove cellular material, and then analyzed for extracellular STXs and geosmin. The second subsample was subjected to three sonication cycles before filtration over 0.45 μm filter to analyze for total STXs and geosmin (both the cell-bound and extracellular compounds). For all STXs and geosmin analysis, confidence limits were calculated using the standard deviations of triplicate measurements. For all experiments the extracellular concentrations of these compounds is always fitted in the confidence limit of total concentration. The difference between total and extracellular levels of any compound was estimated as cell-bound concentration.

In order to study the disinfection by-products (DBP) formation, after 60 minutes of chlorination with the highest chlorine dose (3 mg/L), water samples were quenched with ascorbic acid,

ammonium chloride and sodium thiosulphate for analysis of the THM, HAA and NDMA respectively. Also THM, HAA and NDMA concentrations were measured in control water samples (MR water without CB cells) after 60 minutes of chlorination with 3 mg/L of Cl_2.

2^{nd} Cl_2 Exp: For the STXs extract (STX, C1, C2, GTX2, GTX3) chlorination experiments (no CB cells involved), waters were spiked with the toxin extract to achieve a total STXs concentration of approximately 122 μg/L prior to chlorination. The concentration of 122 μg/L of total STXs was selected in order: (1) to minimize extract related non toxin chlorine demand, (2) to maintain free chlorine residual using relatively low chlorine concentration, e.g. 3 mg/L, and (3) to test a realistic range of STXs concentrations. Control (non-chlorinated) samples were analyzed to confirm the concentration of toxins in test waters after spiking with STXs extract. Chlorine was added from the chlorine stock solution to obtain the desired doses (2 and 3 mg/L). The experiments in ultrapure water were performed at pH 8 maintained by addition of phosphate buffer (0.01 M) (APHA, 1998). Experiments in MR water were performed without pH adjustments (ambient). Samples were quenched at specified contact times with sodium thiosulphate at a stoichiometric ratio (APHA, 1998), and then analyzed for STXs.

Cell Integrity Test. Fluorescein diacetate (FDA) – propidium iodide (PI) staining was chosen to monitor cell viability after chlorination. FDA is transported across the cell membranes and hydrolyzed by cell-bound esterases to produce fluorescein, in metabolically active cells (Hurst et al., 2007). Fluorescein exhibits a green fluorescence when excited by blue light. Propidium iodide (PI) only penetrates cells with damaged membrane, binds to nuclear acids and results in an increase of the red florescence (Hurst et al., 2007).

STXs, Geosmin and DBP Analyses. STXs analyses were conducted using a high performance liquid chromatographic (HPLC) system comprising a 600 pump controller and 717plus autosampler (Waters Pty Ltd, Australia) with postcolumn derivatization and detection using a 2475 multi λ fluorescence detector (Waters Pty Ltd, Australia). The procedures employed were modified from the method of Oshima (Oshima, 1995). Full details are given in the report by the Urban Water Research Association of Australia (Urban Water Research Association of Australia, 1998). Concentrations of the STXs were determined by calibration of the peak areas with that of certified reference standards (Institute of Marine Biosciences, National Research Council, Canada). Samples for geosmin analyses were pre-concentrated using a solid phase

microextraction syringe fiber (Supelco, Australia) and analyzed on a 7890 Gas Chromatograph System with 5975C VL Series Mass Selective Detector (Agilent Technologies, Australia) against quantified labeled internal standards (Ultrafine Chemicals, UK). Full details of this method have been documented by Graham and Hayes (Graham & Hayes, 1998).

THM and HAA analyses were conducted by the Australian Water Quality Center (AWQC) Organic Chemistry laboratory in Adelaide, South Australia; this laboratory is accredited by the Australian National Association of Testing Authorities (NATA). Chlorinated water samples were analyzed for four THM components as total THM (TTHM: bromoform, chloroform, dibromochloroform, and dichlorobromoform) and nine HAA components as HAA9 (bromoacetic, bromochloroacetic, bromodichloroacetic, chloroacetic, chlorodibromoacetic, dibromoacetic, dichloroacetic, tribromoacetic, and trichloroacetic acids). NDMA analyses were conducted by AWQC Applied Chemistry laboratory using gas chromatography-chemical ionization-mass spectrometry following solid-phase extraction.

5.4 Results and Discussion

Impact of A. circinalis cells and STXs extract on chlorine decay. Free chlorine decay and cell integrity experiments were conducted using the A. circinalis culture spiked in MR water at a concentration of approximately 46,000 (±2%) cells/mL at two chlorine doses, 2 and 3 mg/L as Cl2 (Figure 5.1a). The standard deviation value of these cell counts fit within the confidence limit of the cell enumeration method (Cooperative Research Centre for Freshwater Ecology, 1999; Laslett et al., 1997; Lund et al., 1958) (data not shown). CT values were calculated from chlorine decay data by fitting a two pathway integration model to a two phase decay curve: fast (<2 min) and slow (Daly et al., 2007). The chlorine decay model was fitted to each of the decay curves in Figure 5.1a and all correlation coefficients (R2) exceeded 0.90. The resulting first-order rate constants for slow chlorine decay are given in Table 5.1. The apparent rate constants in MR water remained constant regardless of pH and chlorine doses. Cells contribute significantly to chlorine demand and the resulting first-order apparent constants increased accordingly (2.8×10-4 s-1 without cells to 4.6×10-4 s-1 with cells and 2.8×10-4 s-1 without cells to 4.2×10-4 s-1 with cells for 2 and 3 mg/L Cl2 respectively). Increasing chlorine doses from 2 to 3 mg/L at pH 8 does not result in the further release of chlorine reactive cell bound material.

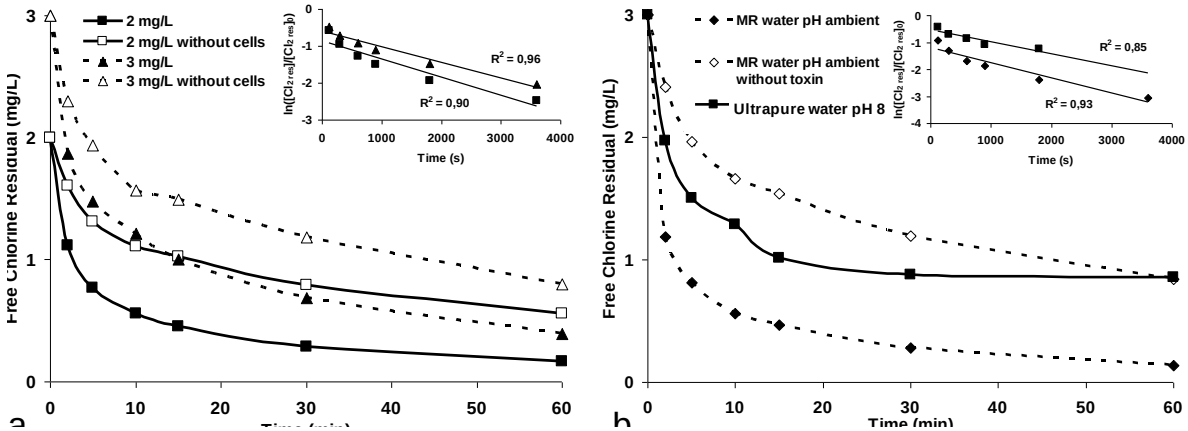

Figure 5.1: Chlorine decay in (a) MR water with and without *A. circinalis* cells at pH 8, (b) MR water with and without STXs extracts and ultrapure water with STXs extracts using 3mg/L Cl_2. Insert: First-order kinetic plots for the slow chlorine decay curves.

Table 5.1: First-order apparent rate constants (k) for slow chlorine decay. Correlation coefficients (R^2) presented in parentheses. (MR- Murray River water)

	k (s^{-1})	
	2 mg/L Cl_2 dose	3 mg/L Cl_2 dose
MR (pH ambient)	-	2.8×10^{-4} (0.93)
MR (pH 8)	2.8×10^{-4} (0.93)	2.8×10^{-4} (0.94)
MR + cells (pH 8)	4.6×10^{-4} (0.90)	4.2×10^{-4} (0.96)
MR + STXs extract (pH ambient)	-	5.6×10^{-4} (0.93)
Ultrapure+ STXs extract (pH 8)	-	4.5×10^{-4} (0.85)

Chlorine decay experiments were also conducted in ultrapure and MR water spiked with 122 µg/L of a STXs extract (no CB cells involved) using a chlorine dose of 3 mg/L Cl_2 (Figure 5.1b). The resulting apparent rate constant value considerably increases with the addition of the STXs extract, clearly showing the presence of chlorine reactive material. Indeed, the background DOC concentration found in MR water increased from 2.4 to 3.7 mg/L following the addition of the STXs extract. Figure 5.1b and Table 5.1 display the impact of pH on results in MR water without cells (pH 8) and with STXs (pH ambient: 6.8) at chlorine dose of 3 mg/L Cl_2 (Figure 5.1).

Effect of chlorination on *A. circinalis* cell integrity and geosmin release in MR water. The impact of chlorination on cell integrity was evaluated using the FDA-PI method. Results from the FDA-PI method were used to classify oxidized cells into two categories of viability:

- "Living active cells" are metabolically active cells with esterase enzymatic reactivity to FDA. These cells have intact membranes that are unlikely to release cell bound geosmin and toxins.
- "Injured/dead cells" includes (1) damaged membrane cells with red florescence (PI) and (2) metabolically inactive cells or no DNA binding sites available (dead cells) (Hurst et al., 2007). Cells in this category have the potential to release geosmin and toxins because of increasing levels of cell membrane damage.

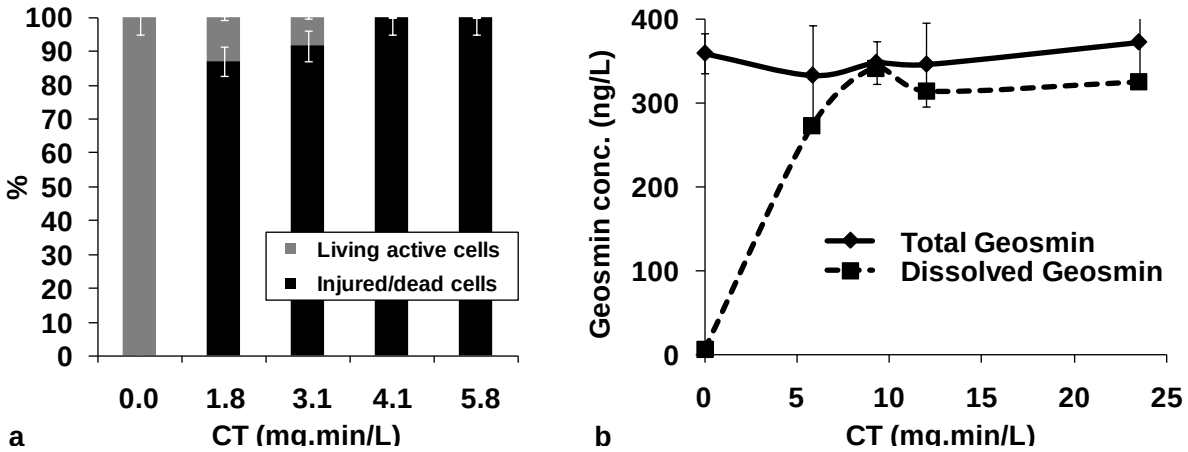

Figure 5.2: (a) Cell viability and (b) geosmin release after chlorination in MR water at pH 8 with 2 mg/L Cl_2 (cell viability results and geosmin release with 3 mg/L Cl_2 follow a similar trend)

The response of two *A. circinalis* suspensions of approximately 46,000 (±2%) cells/mL in MR water to chlorine doses of 2 and 3 mg/L at pH 8 was evaluated. The addition of chlorine to the culture suspensions altered the proportions of cells classified in the living active cells and the injured/dead cells categories. Prior to chlorination 100% of cells were categorized by fluorescence as living active cells. For both chlorine doses this value had decreased to below 2% after a CT of less than 7 mg.min/L (Figure 5.2a). 9% and 15% reduction of total number of cells

was observed for the lower (2 mg/L) and the higher chlorine (3 mg/L) doses, respectively, and the remaining cells were categorized as damaged membrane or metabolically inactive cells.

As chlorination is largely ineffective for geosmin oxidation (Lin et al., 2009), the release of this compound can also be indicative of loss of cell membrane integrity. Figure 5.2b shows the release of geosmin during the chlorination experiment. In the absence of chlorine, nearly all geosmin was cell-bound (>99%). A rapid release of cell bound geosmin was observed after a short exposure to chlorine corresponding to the first quenching time (CTs <10 mg.min/L) (Figure 5.2b). These results are consistent with those from Lin et al. (Lin et al., 2009) observing geosmin release when pre-chlorinating reservoir water loaded with *Anabaena* sp. using low chlorine doses (1-5 mg/L Cl_2). The rapid release of geosmin observed at low CTs (<4.9 mg.min/L, Figure 5.2b) is concurrent with the loss of esterase enzymatic activity (Figure 5.2a). The difference in DOC concentration before and after chlorination was <0.2 mg/L.

In order to describe the kinetics of cell damage, a first-order reaction can be assumed with respect to chlorine reaction and the increase of injured/dead cells (Daly et al., 2007; Lin et al., 2009). Thus it is possible to assume that the reaction between chlorine and the cells is of second order (eq. 5.1).

$$Ln\left(\frac{[N_{CT}]}{[N_0]}\right) = -k_{PI+} \times CT \qquad \text{eq. 5.1}$$

where CT equals the chlorine exposure; N_{CT} equals the number of injured/dead cells after a given chlorine exposure; N_0 equals the number of injured/dead cells at CT=0; and k_{PI+} equals the rate at which cell oxidation occurs (Daly et al., 2007; Lin et al., 2009). However, due to fast kinetics of cell membrane damage caused by chlorine exposure, only estimated rates of 1400 and 1600 $M^{-1}s^{-1}$ were derived for *A. circinalis* cells reaction with 2 and 3 mg/L of Cl_2, respectively. The estimated rates for cell reaction with chlorine from this study are in agreement with the results of Lin et al. (Lin et al., 2009) (*A. circinalis* cell membrane damage rates 1400 - 3400 $M^{-1}s^{-1}$). *A. circinalis* cell damage rate under chlorine exposure was faster than observed rate for a toxic strain of *Microcystis aeruginosa* (670 $M^{-1}s^{-1}$) studied by Daly et al. (Daly et al., 2007). These results demonstrate that *A. circinalis* cells are more vulnerable to chlorine compared with *M. aeruginosa* cells. The susceptibility of cells to oxidation is influenced by the species present, cell

densities, the physiological state of the cells (e.g. the growth phase), water matrix and the conditions of oxidation (Hart et al., 1998; Lin et al., 2009; Pietsch et al., 2002).

Effect of chlorination on cell-bound STXs release and oxidation in MR water. Figure 5.3 shows the total and extracellular toxin concentrations during the chlorination experiments. At both chlorine doses, within a CT of 5 mg.min/L, the cell-bound toxins were completely released. These results are in accordance with rapid geosmin release at CTs <4.9 mg.min/L. The rapid release of the five STXs analogues measured corresponds to the loss of esterase enzymatic activity. For both chlorine doses, cell-bound and extracellular STX were degraded to below detection limit (0.01 µg/L) after a CT of <15 mg.min/L. Oxidation to ~1 µg/L of GTX2, GTX3, C1 and C2 released by the cells required higher CTs (>20 mg.min/L) compared with STX, which had the lowest initial concentration of all released STXs analogues.

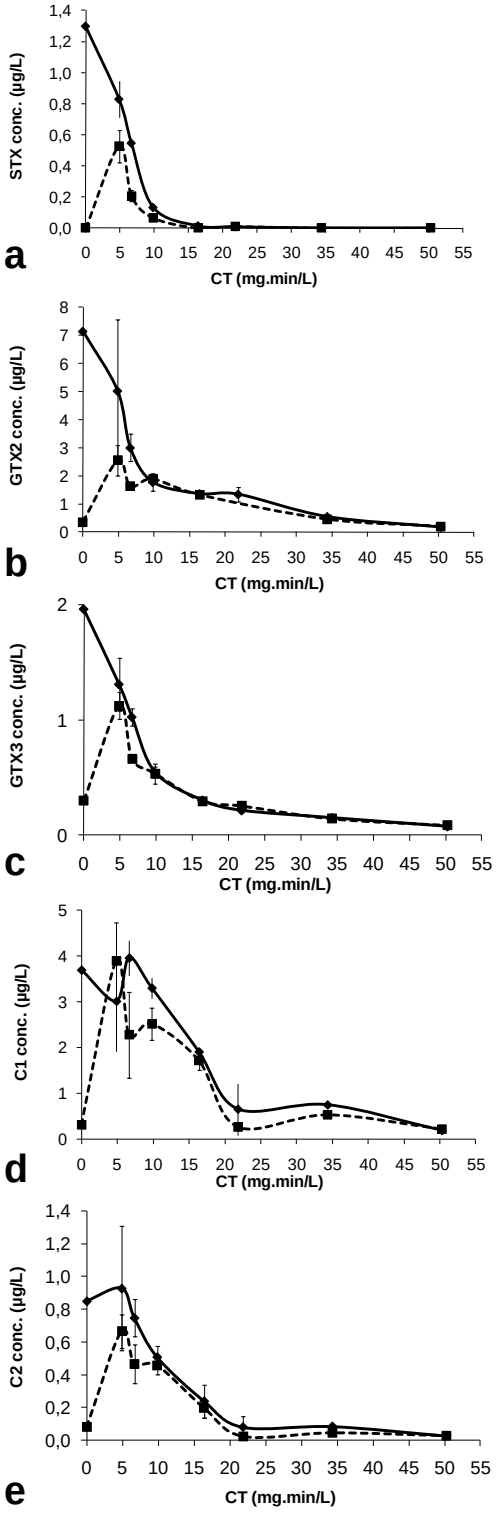

Figure 5.3: Toxin release and oxidation in MR water at pH 8 with 3 mg/L Cl_2 (a) STX, (b) GTX2, (c) GTX3, (d) C1, (e) C2 (Toxin release and oxidation with 2 mg/L Cl_2 follow a similar trend)

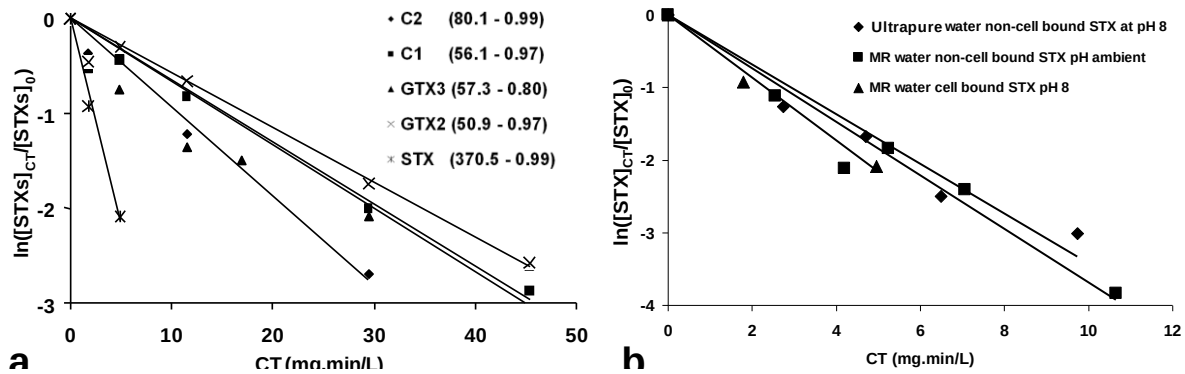

Figure 5.4: Apparent second-order kinetics using 3 mg/L Cl_2 for oxidation of (a) cell-bound STXs in MR water at pH 8 (the values in parentheses are kappt $M^{-1}s^{-1}$ and R^2, respectively), and (b) STX.

Table 5.2: STXs oxidation rate constants (k_{appt}) derived from chlorination experiment using 3 mg/L of chlorine.

STXs variants	k_{appt} ($M^{-1}s^{-1}$)		
	Cell-bound STXs in MR water pH 8	STXs extract in MR water pH 6.8	STXs extract in Ultrapure water pH 8
STX	370.5	322.2	293.5
C1	56.1	-	-
C2	80.1	-	-
GTX2	50.9	-	-
GTX3	57.3	-	-

The order of ease of removal of the STXs with 3 mg/L of chlorine concentration was STX > C2 > GTX3 ~ C1 ~ GTX2 (Figure 5.4a). Very little published information is available on the oxidation of STXs by chlorine. Nicholson et al. (Nicholson et al., 2003) showed that the order of ease of removal by chlorination of dissolved STXs at pH 9 was STX > GTX3 ~ C2 > C1 > GTX2. However, pH was shown to be a critical parameter with differences in STXs susceptibility to chlorine widening below a pH of 8.5 (Cooperative Research Centre (CRC) for Water Quality and Treatment, 2005). The observed pH effect was attributed to ionisation and susceptibility of the various STXs to oxidation by chlorine (Nicholson et al., 2003). More recently, chlorination of STXs at pH 7.3 to 8.0 followed the trend STX > GTX3 ~ C2 > GTX2 ~

C1 (Ho et al., 2009). The trend of chlorine susceptibility revealed from our cell bound STXs oxidation experiment is consistent with previous reports, as STX is the most susceptible analogue, with the trends of the other analogues similar to the previous studies.

The estimated cell membrane damage rate (1400 and 1600 $M^{-1}s^{-1}$) is higher than the STXs degradation rate (370.5 to 50.9 $M^{-1}s^{-1}$). Notably, release of cell-bound STXs due to fast cell membrane damage (Figure 5.2a) was observed prior to their oxidation (below detection limit for STX and 95% for other analogues) by chlorine (Figure 5.3). Daly et al. (Daly et al., 2007) observed that the rate of *M. aeruginosa* cell lysis was approximately three times higher than the MC-LR degradation rate, suggesting that the extracellular component of MC-LR was likely to increase after chlorination.

DBP formation potential of cell bound organic materials and extracellular organic matter (EOM) could be significant if the quantity of reactive carbon associated with the presence of high cell numbers and their EOM is significant (Graham et al., 1998; Huang et al., 2009). Measured concentrations of TTHM and HAA9 as chlorination by-products with the highest CT value (50.3 mg.min/L) were ≤49 µg/L, meeting United States Environmental Protection Agency (USEPA) regulations (80 µg/L for TTHM and 60 µg/L for HAA5) (United States Environmental Protection Agency, 2006). Also, measured NDMA concentration (11 ng/L) with this CT was below the World Health Organization (WHO) guideline value of 0.1 µg/L (WHO, 2008). Furthermore, DBP measurement in control water sample (MR water without cells) with the highest CT demonstrated that >54% of DBP formation was caused by MR water background not CB cells.

DBP yields from cell associated DOC range widely for algae (4 to >350 µg $CHCl_3$/mg DOC) with few but lower values for cyanobacteria (26-50 µg $CHCl_3$/mg DOC for *Anabaena*) (Huang et al., 2009; Nguyen et al., 2005). Reported yields were obtained using intensive culture conditions amenable to carbon accumulation and stringent chlorination dosages that are not representative of natural blooms and plant chlorination practice. The carbon content of our suspension of <47,000 cells/mL *A. circinalis* was estimated from the measured biovolume of 150 μm^3/cell and dry weight of 67.5 pg/cell. Optimum content of carbon (C) in *Anabaena* sp. is ~50% of the cells dry weight (Reynolds, 1984), corresponding to <2 mg/L of cell carbon in the suspensions used. Using the highest published chloroform yield for CB chlorination of 50 µg $CHCl_3$/mg C of

Anabaena sp., a maximum potential formation of <80 µg/L could be expected (Nguyen et al., 2005). Actual THM yield from milder chlorination using the 3 mg/L Cl_2 dose did not produce result in these maximum THM values.

These data provide new information for DW treatment utilities to establish if the toxin release or toxin degradation rate is the limiting step in the complete oxidation of the total STXs. However, these utilities might still face the challenge of odor due to released compounds. These data suggest that pre-chlorination of natural water containing *A. circinalis* at cell numbers ≤47,000 cells/mL for different treatment purposes is a feasible practice while meeting DBP regulations. However, further studies are required to evaluate chlorination potentials of higher cell numbers and of other CB species to better determine the DBP safe zone. In the meantime, high frequency *in vivo* CB monitoring at water intake of treatment utilities could serve as a preventive action to monitor and control the intake into the treatment plant.

The absolute yield and thus the actual mass of potentially reactive natural organic material (NOM) present in the cells is a factor to consider when evaluating whether significant competition for the free oxidant can occur. Thus chlorination experiments with STX extracts were conducted to prove the Cl_2 reaction with cell-bound STX.

Oxidation of STX extract by chlorine in ultrapure and MR waters (no CB cells involved). The oxidation of STX extract in MR water at ambient pH (6.8) and ultrapure water at pH 8 by chlorine was evaluated at a dose of 3 mg/L (Figure 5.1b). Apparent second-order rate constants were determined for the oxidation of STX extract and compared with rate constants for cell bound STX (Figure 5.4b). Slightly faster chlorination of cell-bound STX in MR water (k_{appt}=370.5$M^{-1}s^{-1}$) compared with STX extract in ultrapure water (k_{appt}=293.5$M^{-1}s^{-1}$) at buffered pH was expected reflecting higher NOM concentration in MR water (background DOC of 2.4 mg/L in MR water) (Table 5.2). However, these findings are in accordance with previous observations showing faster toxin degradation rates in natural waters when compared with deionised water (Daly et al., 2007; Ho et al., 2006; Xagoraraki et al., 2006). The reaction of chlorine and NOM could produce species that are more reactive toward toxin than chlorine alone (Ho et al., 2009). Considering the difference between ultrapure and MR water matrix and pH, observed chlorination kinetics for extract and cell-bound STX are very similar (Table 5.2). 2.80×10^{-2} mM Cl_2 and 2.82×10^{-2} mM Cl_2 was consumed for oxidation of ≥99.1% STX extract in

ultrapure water and cell-bound STX in MR water, respectively, using 3mg/L of initial chlorine dose at pH 8; oxidation of STX to below detection limit was achieved in MR water with 5.57×10^{-3}mM Cl_2 surplus.

pH significantly affects the oxidation of STX by Cl_2, oxidation being more effective at pH values over 6.5 (Nicolson et al., 2003). STX contains a purine alkaloid nucleus including nitrogen atoms. The general STX structure has two guanidinium nitrogens of different pKa values. Essentially at higher pH, the degree of protonation of the guanidinium ions is reduced, therefore making the toxin more susceptible to attack at these sites (Cooperative Research Centre (CRC) for Water Quality and Treatment, 2005). The level of STX protonation is more important than the speciation of chlorine between the more reactive hypochlorous acid and the less reactive hypochlorite ion (Cooperative Research Centre (CRC) for Water Quality and Treatment, 2005; Nicholson et al., 2003).

Dissolved and cell bound STXs in water can be oxidized by chlorination. Our study shows that low chlorine doses resulted in the quick release and subsequent oxidation of STXs from a toxic strain of *A. circinalis*. Furthermore, our results showed that toxin release and subsequent oxidation occurred with complete loss of cell viability. To calculate the risk of oxidizing toxic cyanobacterial cells, toxin release and toxin oxidation rates must be quantified. In this case, the detailed investigation of five STXs analogues (STX, C1, C2, GTX2 and GTX3) release and oxidation rates indicated that toxin release rates may not always be the overall removal limiting step. Kinetic analysis of the oxidation of five STXs analogues revealed significant differences in the susceptibility to oxidation by chlorine, STX being the easiest to degrade. Therefore, more attention should be given to provide adequate CT in order to ensure the efficient removal of the recalcitrant analogues after their release. This quantification is especially important for utilities producing unfiltered DW.

5.5 Acknowledgments

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5.6 Supporting Information Available

General structure of geosmin and STXs (Figure SI5-*S1*), geosmin release in MR water with 3 mg/L Cl_2 (Figure SI5.*S2*), cell-bound STXs release and oxidation in MR water with 2 mg/L Cl_2 (Figure SI5.*S3*), measured concentrations of DBP with the highest CT value in MR water (Table SI5.*S4*), and STX extract oxidation with 3mg/L Cl_2 in ultrapure and MR water (Figure SI5.*S5*) are presented in SI. This information is available free of charge via the Internet at http://pubs.acs.org/.

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5.8 Supporting Information (SI)

Release and oxidation of cell-bound saxitoxins during chlorination of *Anabaena circinalis* cells

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Supporting Information (SI)

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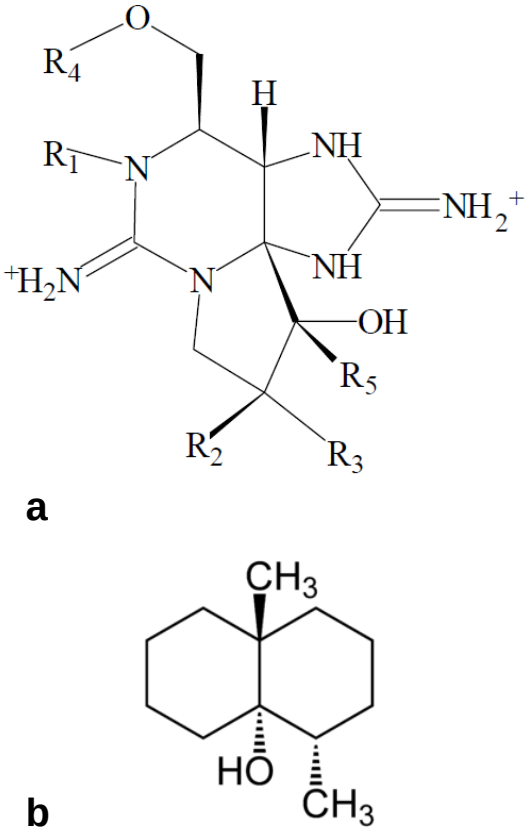

Toxin	R1	R2	R3	R5	Net Charge	Relative mouse toxicity
R4 = $CONH_2$ (carbamate toxins)						
STX	H	H	H	OH	+2	1.000
neoSTX	OH	H	H	OH	+2	0.924
GTX1	OH	H	OSO_3^-	OH	+1	0.994
GTX2	H	H	OSO_3^-	OH	+1	0.359
GTX3	H	OSO_3^-	H	OH	+1	0.638
GTX4	OH	OSO_3^-	H	OH	+1	0.726
R4 = $CONHSO_3^-$ (n-sulfocarbamoyl (sulfamate) toxins)						
GTX5 (B1)	H	H	H	OH	+1	0.064
GTX6 (B2)	OH	H	H	OH	+1	-
C1	H	H	OSO_3^-	OH	0	0.006
C2	H	OSO_3^-	H	OH	0	0.096
C3	OH	H	OSO_3^-	OH	0	0.013
C4	OH	OSO_3^-	H	OH	0	0.058
R4 = H (decarbamoyl toxins)						
dcSTX	H	H	H	OH	+2	0.513
dcneoSTX	OH	H	H	OH	+2	-
dcGTX1	OH	H	OSO_3^-	OH	+1	-
dcGTX2	H	H	OSO_3^-	OH	+1	0.651
dcGTX3	H	OSO_3^-	H	OH	+1	0.754
dcGTX4	OH	OSO_3^-	H	OH	+1	-
LWTX4	H	H	H	H	+2	<0.004
R4 = $COCH_3$ (Lyngbya wollei toxins)						
LWTX1	H	OSO_3^-	H	H	+1	<0.004
LWTX2	H	OSO_3^-	H	OH	+1	0.072
LWTX3	H	H	OSO_3^-	OH	+1	0.021
LWTX5	H	H	H	OH	+2	0.139
LWTX6	H	H	H	H	+2	<0.004
R4 = COC_6H_4OH (Gymnodinium catenatum toxins)						
GC1	H	H	OSO_3^-	OH	+1	-
GC2	H	OSO_3^-	H	OH	+1	-
GC3	H	H	H	OH	+2	-

Figure SI5.*S1*: General structure of (a) STXs* (also known as paralytic shellfish poison) and its known analogues and (b) geosmin.

These neurotoxins can be produced by cyanobacteria (CB)** species including, *Anabaena circinalis*, *Aphanizomenon ovalisporum*, *Cylindrospermopsis raciborskii* and *Lyngbya majuscula* (AFSSA-AFSSET, 2006; Nicholson et al., 2003).

* The STXs molecules degree of ionization is a key factor in oxidation of these toxins. STXs are molecules with a purine alkaloid nucleus which contains nitrogen atoms. The general STXs structure has two guanidinium nitrogens of different pKa values (Cooperative Research Centre (CRC) for Water Quality and Treatment, 2005). The degree of protonation of these nitrogen atoms depends on different factors including the water pH (Nicholson et al., 2003). Also there is a distinct difference in the net charge of these toxins (Figure SI.*S1*), STX has the highest (+2), followed by GTX2 and 3 (+1) and C1 and 2 (0) (Cooperative Research Centre (CRC) for

Water Quality and Treatment, 2005). This charge difference may also influence the degree of toxin molecule protonation in different chlorination conditions (e.g. different pH values). Also, STX has two free guanidinium ions whereas C-toxins have both and GTXs have one of these ions sulphated. This difference in degree of ions sulphatation may be the source of the difference in STXs reaction to chlorine (Cooperative Research Centre (CRC) for Water Quality and Treatment, 2005).

** CB also known as blue-green algae are prokaryotic photosynthetic micro-organisms present in most ecosystems (AFSSA-AFSSET, 2006; Fristachi et al., 2008). Some CB species are producers of a variety of potent toxins (AFSSA-AFSSET, 2006; Chorus & Bartram, 1999; Cramichael et al., 2001, Merel et al., 2010). The increasing frequency and intensity of cyanobacterial proliferation leading to neurotoxin and hepatotoxin production is a universal problem (Chorus & Bartram, 1999; Cramichael et al., 2001, Svrcek & Smith, 2004). Freshwater CB including *A. circinalis* blooms have been found worldwide, including Australia and North American (Merel et al., 2010).

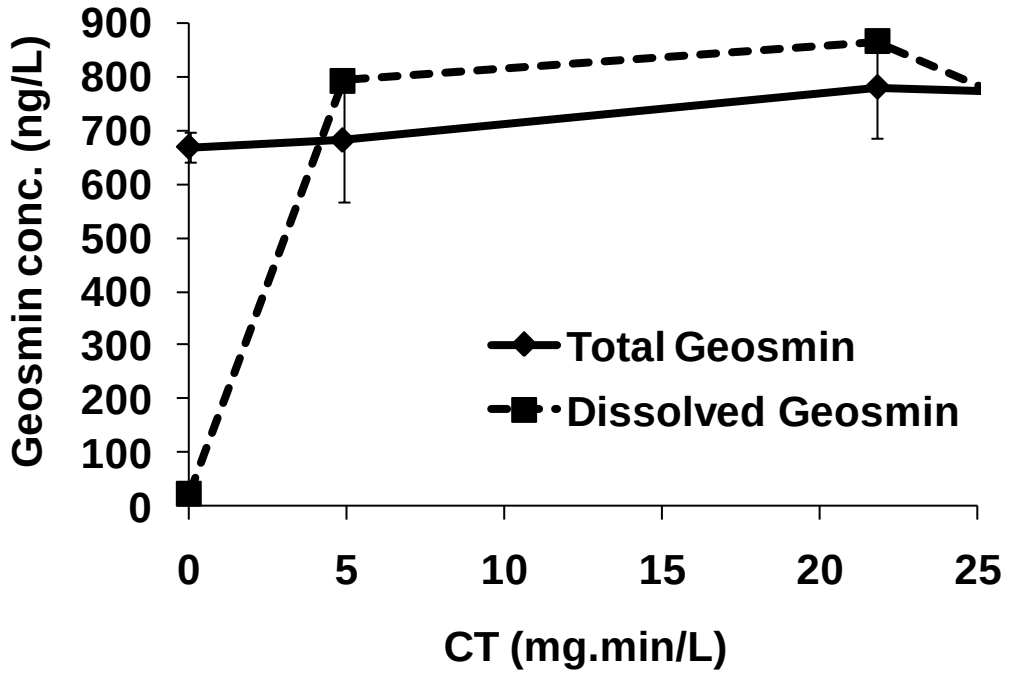

Figure SI5.*S2*: Geosmin release in Murray River water (MR) water at pH 8 with 3 mg/L Cl_2.

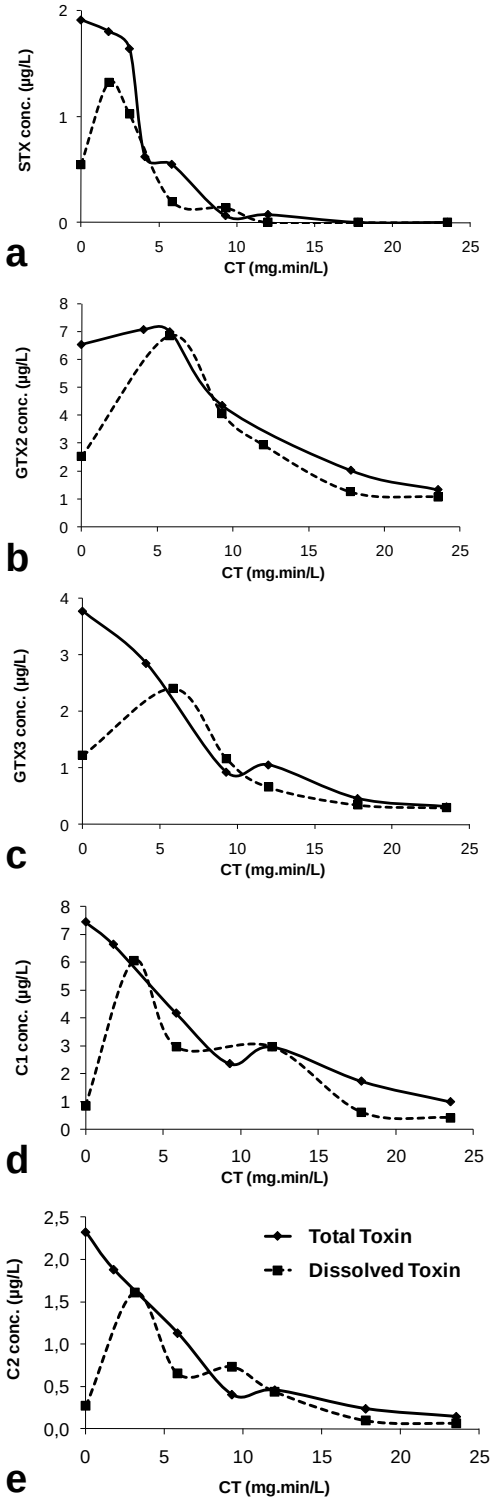

Figure SI5.*S3*: Toxin release and oxidation in MR water at pH 8 with 2 mg/L Cl_2 (a) STX, (b) GTX2, (c) GTX3, (d) C1, (e) C2.

Table SI5.*S4:* Measured concentrations of TTHM, HAA9 and NDMA as chlorination by-products with the highest CT value (50.3 mg.min/L) in MR water without and with *A. circinalis* cells.

Water type	TTHM (µg/L)	HAA9 (µg/L)	NDMA (ng/L)
MR without cells	40	39	6
MR with *A. circinalis* cells	49	42	11

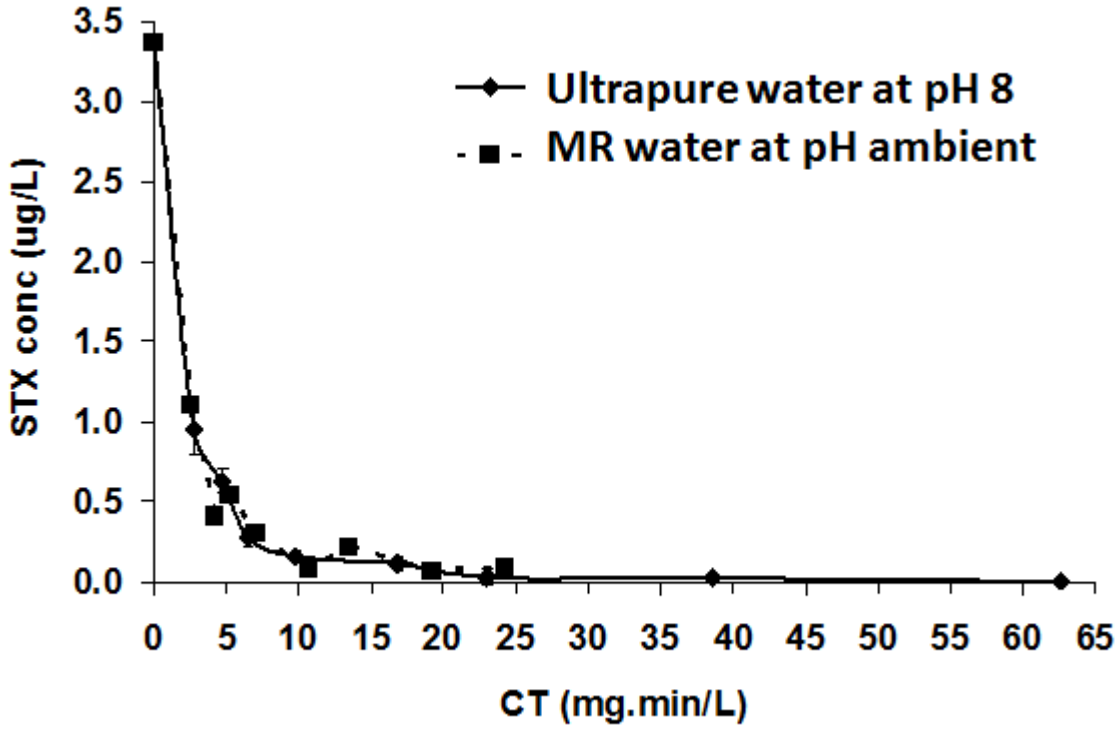

Figure SI5.*S5*: 122 µg/L STX extract oxidation with 3mg/L Cl_2 (a) in ultrapure water at pH 8, (b) in MR river water at pH ambient

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CHAPTER 6 PUBLICATION #3: FATE OF TOXIC CYANOBACTERIAL CELLS AND DISINFECTION BY-PRODUCTS FORMATION AFTER CHLORINATION

This chapter presents the paper published (in press, 2011) in the journal of WATER RESEARCH 2011. This paper titled "Fate of toxic cyanobacterial cells and disinfection by-products formation after chlorination" demonstrates that chlorination of four CB species causes complete cell lysis, cell-bound material including geosmin and three cyanotoxins release, and oxidation of toxins within the disinfection by-products guidelines.

Fate of toxic cyanobacterial cells and disinfection by-products formation after chlorination

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6.1 Abstract

Drinking water sources are increasingly subject to proliferation of toxic cyanobacteria (CB). Direct chlorination of source water containing toxic cyanobacterial cells for different treatment purposes might cause cell damage, toxin release and disinfection by-products (DBP) formation. There is limited information available on chlorination of different toxic CB cells and DBP formation potentials. This work: (1) determines the extent of lysis and toxins/taste and odor compound release in chlorinated natural water from CB cells (*Anabaena circinalis*, *Microcystis aeruginosa, Cylindrospermopsis raciborskii*, and *Aphanizomenon issatsckenka*) from laboratory cultures and natural blooms; (2) assesses the rates of oxidation of toxins by free chlorine under environmental conditions; (3) studies the DBP formation associated with the chlorination of CB cell suspensions. With chlorine exposure (CT) value of <4.0 mg.min/L >60% cells lost viability causing toxin release. Cell-membrane damage occurred faster than oxidation of released toxins. Kinetic analysis of the oxidation of toxins in natural water revealed significant differences in their susceptibility to chlorine, saxitoxins being the easiest to oxidize, followed by cylindrospermopsin and microcystin-LR. Furthermore, concentrations of trihalomethanes and haloacetic acids (<40 μg/L) and N-nitrosodimethylamine (<10 ng/L) as chlorination byproducts were lower than the guideline values even at the highest CT value (220 mg.min/L). However, the DBP concentrations in environmental bloom conditions with very high cell numbers were over the guideline values.

Keywords

Cyanobacteria, cyanotoxins, chlorination, disinfection by-products

6.2 Introduction

The effects of global climate change, particularly changes in water temperature and nutrient loads, appear to enhance the proliferation of potentially toxic cyanobacteria (CB) in drinking water (DW) sources (Elliott et al., 2006; Jöhnk et al., 2008; Paul, 2008). The increasing frequency and intensity of toxic CB blooms in DW sources and detection of cyanotoxins in drinking water treatment plants (DWTP) intakes and treated water is causing several major challenges for DW production and water reuse (Lahti et al., 2001; McQuaid et al., 2011; Merel,

Clément & Thomas, 2010; Svrcek & Smith, 2004). Many cyanotoxins have been identified, with different degrees and mechanisms of human toxicity. Neuro- and hepato-toxicity are the major mechanisms reported to date, with some toxins displaying tumour promoting, and even carcinogenic properties. In addition, some CB species produce several different toxins, and the toxins produced by a particular species can vary between geographical regions (Humpage, 2008; Newcombe et al., 2010; Svrcek & Smith, 2004). The main toxins of interest, the CB species producing these toxins, and their toxicity for humans are summarized in the supplementary data (SD) section (Table SD6.*S1* and Figure SD6.*S2*).

The presence of CB can also cause process disturbances in DWTP using filtration, such as excessive head loss development, short filter runs and increased coagulant demand (Merel, Clément & Thomas, 2010; Mouchet & Bonnelye, 1998). It has been suggested that the accumulation of CB cells in certain treatment processes (e.g. sludge bed of sedimentation tank, surfaces of clarifiers and filters) can be associated with significant toxin release during treatment and raises concerns about the effectiveness of post-oxidation (Pietsch et al., 2002). The release of CB metabolites during treatment has also raised issues of taste and odor (T&O) materials, e.g. geosmin and 2-methylisoborneol (MIB) (SD section: Figure SD6.*S3*), which are aesthetically unpleasant (Hobson et al., 2010; Lin et al., 2009), trihalomethanes (THM), haloacetic acids (HAA) and N-nitrosodimethylamine (NDMA) precursors (El-Dib & Ali, 1994; Graham, Wardlaw et al., 1998), assimilable carbon (Mouchet & Pourriot, 1992), and oxidant demand (Merel, Clément &Thomas, 2010).

In many developing countries, and smaller regional and remote water supplies, efficient filtration processes are not available for DW treatment. This situation could lead to the chlorination of raw water containing potentially toxic CB cells. Furthermore, pre-oxidation ahead of filtration is commonly used in water treatment for many purposes, including the reduction of algal cells and filter cycle optimization (Chen & Yeh, 2005; Mouchet & Bonnelye, 1998). The susceptibility to lysis of CB subjected to chlorination is influenced by the species present, the physiological state of the cells, e.g. the growth phase (Pietsch et al., 2002), and the conditions of oxidation. It has been documented that during chlorination of a cyanobacterial suspension, the rate of toxin release is determined by the rate of the loss of membrane integrity (Daly et al., 2007; Zamyadi et al., 2010).

Chlorine can effectively oxidize different cyanotoxins under specific conditions (Ho et al., 2006; Ho et al., 2009). For the confident application of chlorine to CB cell suspensions, the important parameters to consider are the concentration of chlorine, the CB cells and/or toxin contact time with chlorine and relevant oxidation conditions (pH, temperature, and hydraulic mixing). The exposure to the oxidant is most often expressed in CT, the residual concentration of the oxidant multiplied by time (Ho et al., 2006). Oxidation of the released intracellular toxins will follow the oxidation kinetics established for dissolved toxins, if sufficient residual oxidant is still present at the moment of release (Daly et al., 2007; Zamyadi et al., 2010).

Cell lysis may also lead to a release of cell bound particulate and dissolved organic carbon (DOC) that exert a significant chlorine demand and contribute to the DBP precursor pool (Fang et al., 2010). Laboratory experiments in controlled conditions using culture stocks suggest that oxidant demand from the cells and the released cell bound organics can easily be met with cells numbers <47,000 cells/mL and proper accounting of the chlorine demand of the culture media (Zamyadi et al., 2010). Published results on chlorine demand and DBP formation potential of cyanobacterial suspensions should be assessed in the light of:

- The differences between algal and cyanobacterial cell composition (e.g. cell membrane). Within a particular phylum, the species can vary significantly in terms of their morphology (SD section: Figure SD6.*S4*) and the composition and quantity of excreted organic matter (Henderson et al., 2008).
- The quantity and composition of organic compounds associated with the cells exposed to chlorine. CB cells contain a wide range of intracellular compounds, those released due to autolysis of cells, often referred to as extracellular organic matter (EOM) and those released by compromised lysis, referred to as internal organic matter (IOM) (Fang et al., 2010). The conditions of testing in the laboratory determine the density of cells and the accumulation of EOM, and significant accumulation can occur in batch cultures (Hoyer et al., 1985; Nguyen et al., 2005). EOM release varies widely with the cell growth phase. The organic composition and character of EOM and IOM compounds in CB cells will determine its reactivity to chlorine (Bond et al., 2009; Huang et al., 2009). Furthermore in natural conditions, the background DOC concentrations range widely (1-50 mg/L)

depending on the organic matrix (Thurman, 1985) and these will also have an impact on the chlorine reactivity of the system.

- The concentration of chlorine applied to the cells and used for the DBP testing. Typically industry dosages are low (<1.5 Cl_2:DOC ratio) as opposed to more aggressive dosages applied in laboratory tests (1.7-5 Cl_2:DOC ratio) to oxidize dense cell suspensions and to determine DBP formation potential (Daly et al., 2007; Nguyen et al., 2005; Wardlaw et al., 1991). Typical pre-chlorination doses vary between 0.25-2 mg/l and are adjusted according to water quality parameters (e.g. ammonia concentrations) and the water quality goals to be achieved (e.g. manganese removal and plant hygiene) (AFSSA-AFSSET, 2006).

Table 6.1: Summary of the experimental conditions used by other authors: DBP yields in the literature.

Species	Cl_2 conditions	Chloroform Yield	HAA Yield	production EOM	Comments	Reference
		µgCHCl$_3$/mg TOC	µmoleTHHA/mgTOC	mgDOC/L		
Algae	pH 7 5:1 Cl_2/TOC 24h at 20°C	27	ND	*Not specified*	Intense culture with high DOC	Wardlaw et al. 1991
		378				
		4				
		496				
Cyclotella cells	pH 7 3-5mg/L residual after 7d		ND	1-25	20,000 cells/mL	Plummer and Edzwald 2001
Cyclotella EOM						
Scenedesmus		20				
Cyclotella		50				
Oscillatoria prolifera	pH 7 5:1 Cl_2/TOC 7h at 20oC	30	ND	1-12	Intense culture to maximize the DOC conc. - corr. with UV254	Nguyen et al. 2005
Scenedesmus quadricauda		48				
Anabaena (cell bound)	pH 7 >0.5 mg Cl_2/L after 1-7 days	50	ND	*Not specified*	Intense culture with high DOC and stringent Cl_2 conditions	Huang et al. 2009
Anabaena (EOM)		26				
Microcystis (cell bound)		61	9.73			
Microcystis (EOM)		28	4.61			

Results from limited information on the impact of chlorination of cell-bound toxins and resulting DBP formation are contradictory (Huang et al., 2009; Zamyadi et al., 2010). Table 6.1 summarizes the chlorination conditions and DPB yields reported from various laboratory trials

using cultured strains under a range of chlorination conditions. The diversity of chlorination conditions and the accumulation of EOM in the culture media limit our ability to extend these yields to full scale operations. Consequently, there is a need to better understand the limitations of the oxidation of CB cells and their toxins in natural water, subsequent toxin release and oxidation, and the DBP formation. The objectives of this research were: (1) to determine the extent of lysis and toxins/T&O compounds release in chlorinated natural water from CB cells from laboratory cultures and natural blooms; (2) to assess the rates of oxidation of toxins by free chlorine in environmental conditions; (3) to study the DBP formation associated with the chlorination of CB cell suspensions. To the best of our knowledge, this paper presents the first quantitative study of DBP formation from chlorinated CB cells in natural waters.

6.3 Materials and methods

6.3.1 Chemicals and reagents

Toxic strains of *Anabaena circinalis*, *Microcystis aeruginosa, Cylindrospermopsis raciborskii* and a non toxic strain of *Aphanizomenon issatsckenka* isolated from CB proliferations in Australia were cultured in the laboratory in artificial seawater medium (ASM-1) (Gorham et al., 1964). Distilled water (20 L) was fortified with nutrients according to the ASM-1 preparation, sterilized at 120^{o}C, and after pH adjustment, inoculated with an ASM-1 grown culture. The cultures were incubated at 26^{o}C under 6 hours rotating light-darkness flux (light intensity: 70 μmol s^{-1} m^{-2}).

Toxin spiking experiments were conducted using certified reference materials for saxitoxins (STXs – STXs analogues are saxitoxin (STX), C-toxin1 (C1), C-toxin2 (C2), gonyautoxin2 (GTX2), and gonyautoxin3 (GTX3)) and microcystin-LR (MC-LR) purchased from the Institute of Marine Biosciences, National Research Council of Canada. MC-LR certified material was also used as standards for qualification/quantification purposes. All chemicals and reagents were analytical grade from various suppliers.

6.3.2 Water sources

Water for the oxidation tests with the laboratory cultures was sampled from Prospect reservoir in New South Wales, Australia. Samples were collected from the intake to the Prospect DWTP

prior to any treatment. Raw Prospect water was filtered over pre-rinsed Binder-free glass microfiber 1.2µm filter (GF/C - Whatman, England) prior to inoculation with culture. The water had a DOC concentration of 3.9 mg/L, UV absorbance (at 254 nm) of 0.066 cm^{-1}, pH of 7.6, alkalinity of 79 mg/L as $CaCO_3$, free ammonia was below the detection limit (<0.1 mgNH_3/L). An environmental CB bloom sample was taken from the Torrens River (TR) in Adelaide, South Australia (SA) in March 2010. Several recreational activities are conducted on the river which crosses downtown Adelaide. In that water, CB cell numbers were estimated at 5.5×10^6 cells/mL by contrast microscopy, while the DOC concentration was 9.7 mg/L, and pH at 8.7. Prior to DOC analyses, samples were passed through pre-rinsed 0.45 µm cellulose nitrate membrane filters (Schleicher and Schuell, Germany). DOC measurements were made on an 820 total organic carbon analyzer (Sievers Instruments Inc., USA). pH was measured on a PHI 50pH meter (Beckman Instruments, USA) which was calibrated with pH 4, 7 and 10 standard buffers (BDH, Australia).

6.3.3 Chlorination experiments

A chlorine stock solution was prepared by bubbling gaseous chlorine through high purity water in a glass flask which was sealed and stored at 4°C for at least 16 hours prior to application (Ho et al., 2006). Chlorine stock and chlorine residual concentrations were determined using the DPD-FAS titration method described in Standard Methods (American Public Health Association et al., 1998). Typical chlorine stock solutions concentrations ranged from 2000-6000 mg/L as free chlorine. The experimental plan included chlorination of (1. CB cultures) STXs producing *A. circinalis*, MC-LR producing *M. aeruginosa*, cylindrospermopsin (CYL) producing *C. raciborskii*, and *A. issatsckenka* suspensions and (2. CB bloom) a sample of CB bloom from TR. A chlorine decay experiment was conducted prior to each experiment. For chlorine decay experiments, water samples were dosed with the desired chlorine concentration and triplicate samples taken at specified contact times for free chlorine residual determinations using the DPD-FAS titration method. All chlorination experiments were conducted in triplicate in brown amber bottles at room temperature (20 ± 2°C).

1. CB cultures: Cultured CB cells were enumerated on a compound microscope in a Sedgewick-Rafter counting chamber after preservation in Lugol‟s iodine (Cooperative Research Centre for Freshwater Ecology, 1999). The required volume of culture at early stationary phase to be spiked

in Prospect water to achieve the desired cell number was adjusted based on the cell number in the culture. The number of cells in Prospect water after inoculation was confirmed by microscopic count. Unchlorinated water samples showed no sign of cell integrity loss due to direct addition of cells to Prospect water (data not shown). For the extracellular toxins chlorination experiments, MC-LR and STXs standard extracts were spiked to the *M. aeruginosa* and *A. circinalis* suspensions, respectively, to achieve concentrations of approximately 15 µg/L and 3 µg/L of saxitoxin (STX) equivalent (eq), in addition to any intracellular toxin concentrations prior to chlorination. The concentrations of extracellular toxins were selected in order to: (a) maintain free chlorine residual using relatively low chlorine concentration, e.g. 2 mg/L, and (b) test a realistic range of intra- and extracellular toxin concentrations. Unchlorinated samples were analyzed to confirm the concentration of toxins in test waters after spiking with toxin extract. *2. CB bloom*: Environmental CB bloom samples were taken from TR (5.5×10^6 cells/mL) where the CB population was counted for 98% *Microcystis flos-aquae* and 2% *Anabaena circinalis*.

The pH of water samples were maintained at 8 by the addition of phosphate buffer (0.01 M) (American Public Health Association, 1998). Chlorine was added from the chlorine stock solution to obtain the desired doses (2 and 5 mg/L). Samples were quenched at specified contact times with sodium thiosulphate at a stoichiometric ratio specified in Standard Methods (American Public Health Association, 1998). Quenched samples were taken for DOC measurement. To determine cell viability two microscopic counts were conducted in triplicate: (1) cell integrity tests were conducted within 30 minutes after the end of experiment on non-preserved 2 mL samples, and (2) total cell counts on 2 mL samples after preservation in Lugol‟s iodine (Cooperative Research Centre for Freshwater Ecology, 1999). Remaining quenched water samples were divided in two subsamples of 250 mL: the first subsample was immediately filtered through 0.45 µm filter (Whatman, UK) to remove cellular material, and then analyzed for extracellular toxins only in the case of *C. raciborskii* and geosmin/MIB only in case of *A. circinalis* and TR bloom samples. The second subsample was subjected to three sonication cycles before filtration over 0.45 µm filter to analyze for total toxins and geosmin/MIB (both the cell-bound and extracellular compounds). For all experiments the extracellular concentrations of all toxins and geosmin/MIB analysis were always below the total concentration of these

compounds. The difference between total and extracellular levels of any compound was estimated as the cell-bound concentration.

In order to study the DBP formation after 60 minutes of chlorination, water samples were quenched with ascorbic acid, ammonium chloride and sodium thiosulphate for analysis of THM, HAA and NDMA, respectively. Concentrations of these compounds were also measured in control water samples (Prospect water without CB cells) after 60 minutes of chlorination.

6.3.4 Cell integrity test

Cell viability after chlorination was monitored using Fluorescein diacetate (FDA) – propidium iodide (PI) staining (Hurst et al., 2007). FDA is transported across the cell membranes and hydrolyzed by cell-bound esterases to produce fluorescein, in metabolically active cells. Fluorescein exhibits a green fluorescence when excited by blue light under the microscope. PI only infiltrates damaged cells membrane, binds to nuclear acids and causes an increase of red fluorescence under the microscope.

6.3.5 Analysis of toxins, geosmin/MIB and DBP

MC-LR analyses were conducted using a high performance liquid chromatographic (HPLC) system comprising a 600 pump controller, 717plus autosampler and 996 photodiode array detector (Waters Pty Ltd, Australia) using methods described by Ho et al. (2006). Concentrations of the MC-LR were determined by calibration of the peak areas with that of certified reference standards. STXs and CYL analyses were conducted using an Abraxis Saxitoxin ELISA Plate (an enzyme-linked immunosorbent assay – Abraxis LLC, Pennsylvania, USA) and a Beacon Cylindrospermopsin Plate kit (a competitive enzyme-labeled immunoassay – Beacon Analytical Systems Inc. Maine, USA), respectively. The analyses of STXs concentrations were measured as µg/L of STX eq. The accuracy of these Plates was validated using standard toxin materials (SD section: Table SD6-*S5*). Samples for geosmin/MIB analyses were pre-concentrated using a solid phase microextraction syringe fiber (Supelco, Australia) and analyzed on a 7890 Gas Chromatograph System with 5975C VL Series Mass Selective Detector (Agilent Technologies, Australia) against quantified labeled internal standards (Ultrafine Chemicals, UK). Full details of this method have been documented by Graham and Hayes (1998).

THM and HAA analyses were conducted by the Australian Water Quality Center (AWQC) in Adelaide, SA; this laboratory is accredited by the National Association of Testing Authorities, Australia (NATA). Chlorinated water samples were analyzed for bromoform, chloroform, dibromochloroform, and dichlorobromoform, THM components as total THM (TTHM) and bromoacetic, bromochloroacetic, bromodichloroacetic, chloroacetic, chlorodibromoacetic, dibromoacetic, dichloroacetic, tribromoacetic, and trichloroacetic acids, HAA components as HAA9. NDMA analyses were conducted using a gas chromatography-mass spectrometry (GCMS) system (Agilent Technologies, Australia) operated in positive chemical ionization mode. The method was redeveloped and optimized from a previously adapted method (Schmidt et al., 2006).

6.3.6 Data analysis

For the chlorination experiment where the reaction between chlorine and the cells or toxins is of second order, equation 1 was used to calculate the chlorination apparent second-order rate constants:

$$Ln\left(\frac{[X_{CT}]}{[X_0]}\right) = -k \times CT \qquad \text{Equation 1}$$

where CT equals the chlorine exposure; X_{CT} equals the number of injured/dead cells or toxin concentration after a given chlorine exposure; X_0 equals the number of injured/dead cells or toxin concentration at CT=0; and *k* equals the rate at which cell (k_{PI+}) or toxin (k_{appt}) oxidation occurs (Daly et al., 2007; Lin et al., 2009).

6.4 Results and discussion

6.4.1 Impact of CB cells, intra and extracellular toxins on chlorine decay

Free chlorine decay and cell integrity experiments were conducted using the CB cultures spiked in Prospect water at cell numbers of approximately 50,000 and 200,000 (±2%) cells/mL and TR bloom samples at cell number of approximately 5.5×106 (below ±5%) cells/mL, at two chlorine doses, 2 and 5 mg/L as Cl2 (Figures 6.1a & b). The standard deviation value of these cell counts (triplicate count per sample) fit within the confidence limit of cell enumeration method (Cooperative Research Centre for Freshwater Ecology, 1999; Laslett et al., 1997; Lund et al.,

1958) (data not shown). The chlorine decay in Prospect water in the absence of cells was lower than in the presence of cells by approximately 1.2 mg/L. The chlorine decays in the presence of cells were similar for all CB species. CT values were calculated from chlorine decay data by fitting a two pathway integration model to a two phase decay curve: fast (<2 minutes) and slow (Sohn et al., 2004). The resulting first order rate constants for chlorine decay are given in Table 6.2. The apparent rate constants in Prospect water without cells were very stable and increased slightly with the chlorine doses and the addition of cells (Table 6.2). No fixed trends were observed between species tested as a function of chlorine dosage.

Chlorine decay experiments were also conducted in TR bloom samples using the same chlorine doses (Figure 6.1b). Higher apparent rate constant values were observed compared to the Prospect water suspension, a consequence of the elevated cell numbers and elevated DOC of 9.7mg/L. The resulting apparent rate constant value at 2mg/L probably is an underestimation as chlorine residuals were minimal after 15 minutes (Table 6.2).

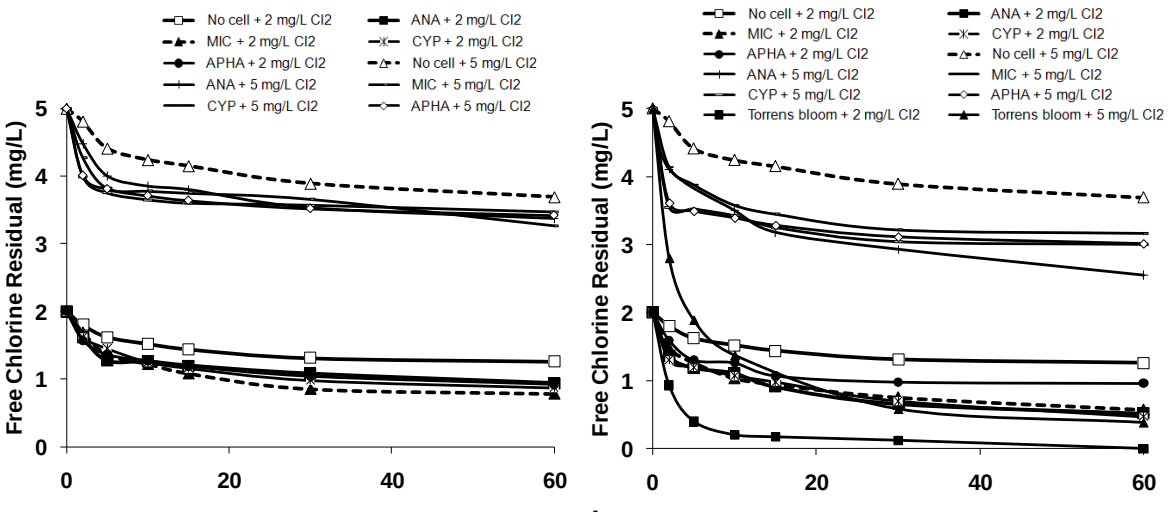

Figure 6.1: Chlorine decay in (a) Prospect water without and with 50,000 cells/mL CB at pH 8, (b) Prospect water without and with 200,000 cells/mL CB and Torrens (TR) bloom water samples with 5.5×10^6 cells/mL at pH 8.

Table 6.2: First-order apparent rate constants (k) for chlorine decay. All correlation coefficients (R^2) exceeded 0.84.

Source of water/bloom	CB species	50,000 cells/mL		200,000 cells/mL	
		2 mg/L Cl_2	5 mg/L Cl_2	2 mg/L Cl_2	5 mg/L Cl_2
Prospect Water	No cell	3.4×10^{-4}	4.2×10^{-4}	3.4×10^{-4}	4.2×10^{-4}
	A. circinalis	1.5×10^{-3}	7.3×10^{-4}	1.7×10^{-3}	5.0×10^{-4}
	M. aeruginosa	1.3×10^{-3}	8.8×10^{-4}	1.5×10^{-3}	8.1×10^{-4}
	C. raciborskii	1.0×10^{-3}	9.1×10^{-4}	1.6×10^{-3}	1.1×10^{-3}
	A. issatsckenka	1.3×10^{-3}	8.6×10^{-4}	1.0×10^{-3}	1.1×10^{-3}
bloom	*Microcystis* + *Anabaena*	5.5×10^6 cells/mL			
		3.7×10^{-3}	2.0×10^{-3}		

6.4.2 Chlorination effect on CB cell integrity and geosmin/MIB release in Prospect and TR bloom water.

The FDA-PI method was used to assess the impact of chlorination on cell integrity. Thus chlorinated cells were classified into two categories of viability (Hurst et al., 2007):

- "Living active cells" are metabolically active cells with intact membranes that are unlikely to release cell bound toxins and T&O compounds.
- "Injured/dead cells" have (1) damaged membranes (red florescence due to PI penetration) and (2) metabolically inactive or no DNA binding sites available (dead cells). Cells in this category have the potential to release toxins and odor compounds due to cell membrane damage.

The response of eight CB suspensions of approximately 50,000 and 200,000 (±2%) cells/mL in Prospect water to chlorine doses of 2 and 5 mg/L at pH 8 was evaluated. Chlorination of the CB suspensions changed the proportions of cells classified in these two categories. Before chlorination 100% of cells were categorized using fluorescence as living active cells (Figure 6.2). In the case of *A. circinalis*, *C. raciborskii*, and *A. issatsckenka* for both chlorine doses, this value had decreased to below 1% after a CT of less than 8 mg.min/L (Figures 6.2a, c & d). However, in the case of *M. aeruginosa* a CT of 31 mg.min/L was required to damage >99% of the cells (Figure 6.2b). For all CB species 60% to 80% reduction of total number of cells, corresponding to a full lysis was observed at the end of the chlorination period for both chlorine doses. The remaining cells were categorized as damaged membrane or metabolically inactive cells (Figure 6.2).

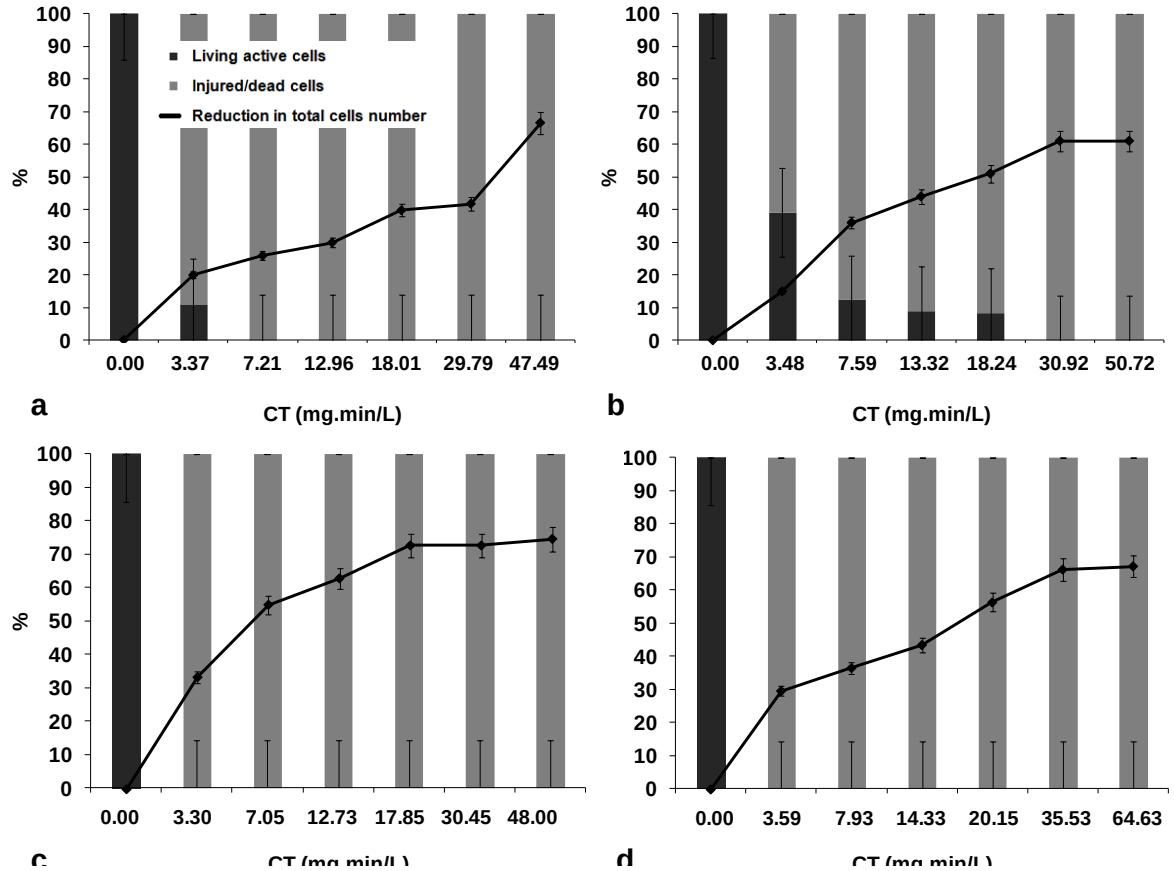

Figure 6.2: Cell viability after chlorination of 200,000 CB cells in Prospect water at pH 8 with 2 mg/L Cl_2 (a) *Anabaena circinalis*, (b) *Microcystis aeruginosa*, (c) *Cylindrospermopsis raciborskii* and (d) *Aphanizomenon issatsckenka* (cell viability results with 2 and 5 mg/L Cl_2 and other cells number is following a similar trend).

As chlorination is ineffective for geosmin oxidation (Lin et al., 2009) geosmin release can also be indicative of loss of cell membrane integrity. In the absence of chlorine, over 96% of the *A. circinalis* geosmin was cell-bound. A fast release of cell bound geosmin was observed after a short exposure to chlorine, 89 and 100% extracellular geosmin were observed at CTs of 3.4 and 7.2 mg.min/L, respectively. These results are consistent with previous studies (Lin et al., 2009; Zamyadi et al., 2010) who observed geosmin release when pre-chlorinating (with low chlorine doses, 1-5 mg/L Cl_2) natural water loaded with *Anabaena* sp. The fast release of geosmin with low CTs (<8 mg.min/L) is simultaneous with the loss of cell integrity (Figure 6.2a). DOC

variations before and after chlorination were <0.3 mg/L. Similar results were observed in chlorination of bloom samples from TR. Dissolved geosmin and MIB concentration in bloom samples before chlorination were 61 and 49 ng/L, respectively. With a CT of <5.6 mg.min/L, 100 and 67% release of cell-bound geosmin and MIB was observed, respectively. Maximum values of 716 and 124 ng/L were observed after CT of 21.3 mg.min/L for dissolved geosmin and MIB.

It is possible to assume a first-order reaction with respect to chlorine concentration and the increase in the number of injured/dead cells (Daly et al., 2007; Lin et al., 2009). Thus the reaction between chlorine and the cells is assumed to be of second order and the rate at which cell oxidation occurs (k_{PI+}) is evaluated using equation 1. However, in this experiment, due to the fast kinetics of cell membrane damage due to chlorination only estimations of rates of CB cells reaction with 2 and 5 mg/L of Cl_2 were possible (Table 6.3). The estimated rates for cell reaction with chlorine from this study are in broad agreement with the results of previous studies (Table 6.3). These results demonstrate that *C. raciborskii* and *A. issatsckenka* are the most vulnerable CB cells to chlorination. *A. circinalis* cells are more vulnerable to chlorine compared with *M. aeruginosa* cells. The vulnerability of the cells of the CB species to chlorination is influenced by the physiological state of the cells, cell densities, water matrix and the chlorination conditions (Lin et al., 2009; Pietsch et al., 2002).

Table 6.3: Cyanotoxins kappt ($M^{-1}s^{-1}$) derived from this study compared to published results. Comments in parenthesis describe the origin of the CB cells (from laboratory cultures or environmental bloom samples) and the toxins (from standard extracts or cell-bound).

	50000 cells/mL 2 mg/L Cl_2	50000 cells/mL 5 mg/L Cl_2	200000 cells/mL 2 mg/L Cl_2	200000 cells/mL 5 mg/L Cl_2	Acero et al., 2005	Ho et al., 2006	Rodriguez, Onstad et al., 2007; Rodriguez, Sordo et al., 2007b	Daly et al., 2007	Lin et al., 2009	Zamyadi et al., 2010
pH	This study at pH 8				7.9-8	7.9	8	6.8-7.6	8.3-8.6	6.8-8
CB species										
A. circinalis	1930	2070	1607	1690					1400-3400	1400-1600
M. aeruginosa	980	1030	756	820				670±77	790-1100 (culture), 70-590 (bloom)	
C. raciborskii	4050	4106	3593	3890						
A. issatsckenka	4105	4190	3303	3882						
Toxin										
STX	170.9*	69.3*	132.6*	51.5*						370.5 (cell-bound), 293.5-322.2 (extract)
C 1 & 2, GTX 3 & 4										50.9-80.1 (cell-bound)
MC-LR	21.1	21.6	21.0	23.3	33.1-38.0	110-170	33 (extract)	242 (extract), 10-96 (cell-bound)		
CYL**	186.4	8.2	47.0	18.1			490 (extract)			

* STX eq.: Samples analyzed using Abraxis Saxitoxin ELISA Plate in µg/L of STX equivalent and do not differentiate between the different analogues of STXs.

** Estimate k values from 3 points values.

6.4.3 Chlorination effect on cell-bound and extracellular toxins release and oxidation in Prospect water samples

Figures 6.3a & b and Figure 6.4 show the total and extracellular toxin concentrations during the chlorination experiments. In the case of *C. raciborskii* at both chlorine doses, within a CT of 3.3 mg.min/L, the cell-bound CYL was completely released (Figure 6.4). Additionally, the fast geosmin release at very low CTs (<3.4 mg.min/L) is an indication of complete release of cell-bound STXs (Zamyadi et al., 2010). The fast release of the toxins corresponds to the loss of cell integrity (Figure 6.2). For both chlorine doses and *A. circinalis* cell numbers cell-bound and extracellular STXs were degraded to <1µg/L after a CT of 80 mg.min/L. However, the STXs concentrations remained constant for the rest of the chlorination period (Figure 6.3a). Chlorination of cell-bound and extracellular MC-LR for both *M. aeruginosa* cell numbers using 2 and 5 mg/L Cl_2 resulted in 73% and 91% degradation of toxin, respectively (Figure 6.3b). Limited measurements of CYL decay in *C. raciborskii* suspensions show CYL removals ranging from 83% to 99% in different chlorination experiments (Figure 6.4). These results are consistent with a recent study observing fast and significant decrease of CYL toxicity due to chlorination (Cheng et al., 2009; Merel, Clément, Mourot et al., 2010). Toxin production of *M. aeruginosa* and *C. raciborskii* were not proportionally reflected in the experiments with higher cell numbers.

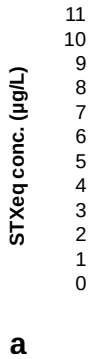

Figure 6.3: Toxin oxidation in Prospect water at pH 8 with 2 and 5 mg/L Cl_2 (a) STX equivalent in *Anabaena circinalis* cell suspensions, and (b) MC-LR in *Microcystis aeruginosa* cell suspensions.

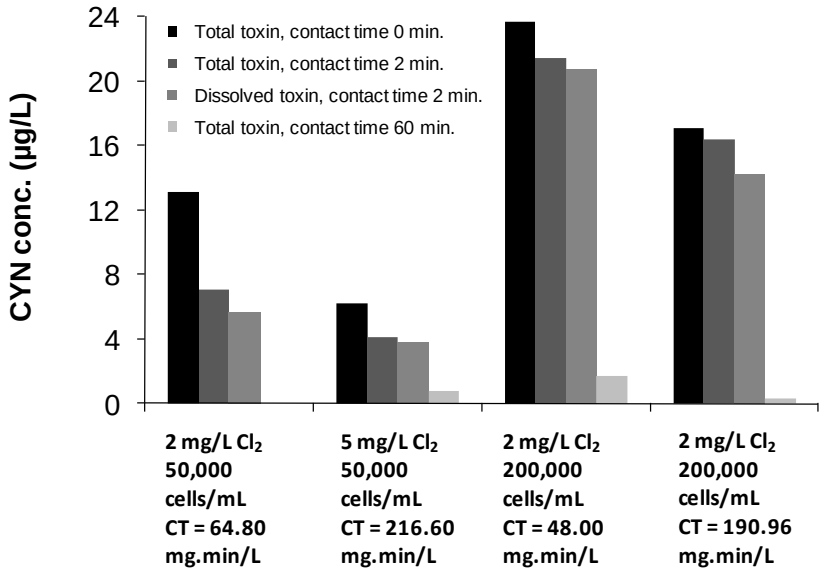

Figure 6.4: CYL release and oxidation in *Cylindrospermopsis* cell suspensions in Prospect water at pH 8 with 2 and 5 mg/L (the CT values given on the *x* axis are the CT values calculated after the 60 minute of contact).

Kinetic models have been used to express the oxidation of released MC-LR, CYL, and STXs from cells following chlorination (Daly et al., 2007; Rodriguez, Sordo et al., 2007; Zamyadi et al., 2010). These models were based on the apparent rate constant for toxin chlorination and the decreasing chlorine concentration in the water. A first-order reaction is assumed with respect to chlorine and the concentration of the toxins. Hence a second order reaction is assumed between chlorine and the toxins. Using the CT values and the rate constants, the toxin concentration can be calculated using equation 1, where k_{appt} is the apparent second-order rate constant for the reaction of the chlorine with the toxin under consideration at a given pH. Results derived from values obtained at 2 and 5mg/L of Cl_2 (Figures 6.3 and 6.4) were used to estimate toxins k_{appt} (Table 6.3).

All CT experiments in Prospect water were conducted using excess chlorine (with the presence of Cl_2 residual). The conditions used are considered representative of practice in DWTP. k_{appt} were determined for the oxidation of toxin extracts and compared with documented rate constants for cell bound toxins at different pH (Table 6.3). The order of ease of oxidation of the STXs with chlorine has been published, with STX being the easiest to oxidize, followed by

C1&2 and GTX2&3 (Nicholson et al., 2003; Zamyadi et al., 2010). pH significantly affects the oxidation of MC-LR by Cl_2, oxidation being more effective at pH below 8. The observed pH effect was attributed to protonation of MC-LR making it more vulnerable to chlorination (Ho et al., 2006). k_{appt} of MC-LR chlorination at pH 8 in the presence and absence of cells is in the range of 21-170 $M^{-1}s^{-1}$ (Table 6.3) (Rodriguez, Onstad et al., 2007). The presence of CB cells had a major impact on chlorination of CYL as the oxidation reaction was lower in cell-bound CYL (this study: 8.2-186.4 $M^{-1}s^{-1}$) compared with CYL extract (Rodriguez, Sordo et al. (2007): 490 $M^{-1}s^{-1}$) (Table 6.3).

The estimated *A. circinalis* and *M. aeruginosa* cell membrane damage rates are in accordance with previous observations (Table 6.3) showing faster cell membrane damage than the degradation of the respective toxins. Notably, the release of cell-bound toxins due to fast cell membrane damage was observed prior to their chlorination suggesting that the extracellular component of toxins was likely to increase after chlorination (Daly et al., 2007; Zamyadi et al., 2010).

The chlorine demand of identified cell components and the resulting DBP formation varies widely (Bond et al., 2009). Nitrogenous compounds are very reactive with chlorine and form DBPs such as THM, HAA and/or NDMA (Hureiki et al., 1994; Bond et al., 2009). Other key components of EOM and IOM such as carbohydrates are not reactive with chlorine and form minimal quantities of THM and HAA (Deborde & von Gunten, 2008; Bond et al., 2009). A focus of our paper is to demonstrate if the oxidation of cell-bound and extracellular toxins can be achieved in CB suspensions under mild chlorination conditions typical of those applied by industry and study the DBP formation under chlorination conditions.

DBP formation potential of IOM and EOM could be significant with a major quantity of reactive carbon associated with the presence of high cell numbers and their EOM (Graham, Wardlaw et al., 1998; Huang et al., 2009). Measured concentrations of TTHM and HAA9 with the highest CT value (for both Cl_2 doses in Prospect water) were ≤40 µg/L (Figure 6.5), meeting United States Environmental Protection Agency (USEPA) regulations (80 µg/L for TTHM and 60 µg/L for HAA5) (United States Environmental Protection Agency, 2006). Also, the measured NDMA concentration (11 ng/L) with this CT (Figure 6-5a & c) was below the World Health Organization (WHO) guideline value (0.1 µg/L) (WHO, 2008). Additionally, DBP measurement

in the control water sample (Prospect water without cells) with the highest CT established that ≥52% of DBP formation was caused by the organics in Prospect water and not CB cells. However, the DBP formation in TR water samples was close to or exceeded the guideline values mainly due to high DOC concentrations (Figure 6.5b & d).

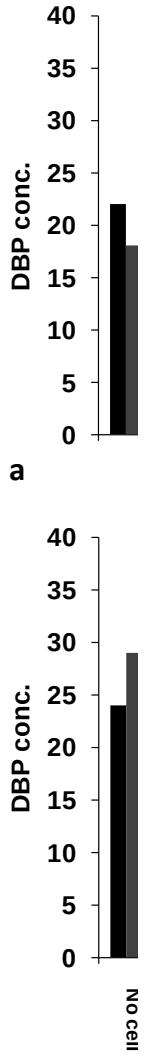

Figure 6.5: Disinfection by products formation at pH 8 and 60 minutes of contact time with 2 mg/L Cl_2 in (a) Prospect water, (b) TR bloom, and 5 mg/L Cl_2 in (c) Prospect water and (d) TR bloom.

The CB cells used were harvested at early stationary phase of growth. EOM release also varies widely with the cell growth phase and can reach extreme values of 40mg/L in very dense cell suspension (Hoyer et al., 1985; Nguyen et al., 2005). In the case of CB, while EOM concentrations increase from the onset of growth to early stationary phase, the composition of the EOM varies. During the exponential growth phase, low molecular weight intermediate products such as glycolic and amino acids are dominant, while EOM from senescent cells are typically composed of high molecular weight products, such as polysaccharides (Nguyen et al., 2005). Thus, EOM excreted mainly during the exponential phase of growth has been shown to be very reactive to chlorine and a precursor to DBP in some cases (Huang et al., 2009; Nguyen et al., 2005). However, many of the experiments in which this high reactivity was observed were conducted in the laboratory using protocols designed to maximize EOM production with elevated concentrations produced (>10mg/L) using very dense monocultures (>0.5mg/L Chl*a*) and extreme chlorination conditions (Table 6.1).

Table 6.4: Estimated cell carbon equivalent to CB cell number of this work and potential THM formation (ANA: *A. circinalis*, MIC: *M. aeruginosa*, CYP: *C. raciborskii* and APHA: *A. issatsckenka*).

CB	Estimated biovolume (μm^3/cell)	Max. estimated dry weight (DW) (pg/cell)	Max Carbon cell mass (max 50% DW)	Nb. cells/L in Cl_2 assay	Cell C mass (mg/L)	Yield $\mu gCHCl_3/mgC$	Potential TTHM Yield (µg/L)
ANA	76	49*	24.5	50000000	1.23	50.0	62.3
ANA	76	49*	24.5	200000000	4.90	50.0	245.0
MIC	53	44*	22.0	50000000	1.10	61.0	67.1
MIC	53	44*	22.0	200000000	4.40	61.0	268.4
CYP	58	10.2**	5.1	50000000	0.26	61.0***	15.6
CYP	58	10.2**	5.1	200000000	1.02	61.0***	62.2
APHA	24	4.2*	2.1	50000000	0.11	61.0***	6.4
APHA	24	4.2*	2.1	200000000	0.42	61.0***	25.6

* Reynolds (1984)

** Estimated based on cell biovolume

*** No yield data available, the maximum yield for CB cells (Table 2) were used

Cell bound DOC may constitute a significant source of DBP precursors in some situations based on the DBP yields available in the literature (Tables 6.1 and 6.4). DBP yields from cell

associated DOC have a broad variation for algae (4 to >350 µg$CHCl_3$/mgDOC) with few lower values for CB (26-50 µg$CHCl_3$/mgDOC for *Anabaena*) (Huang et al., 2009; Nguyen et al., 2005). A more recent study suggests that the reactivity and specific by-product formation potential are similar for various NOM and IOM from laboratory cultured *Anabaena* and *Microcystis* (Huang et al., 2009). In conclusion the reactivity and DBP formation potential of IOM and EOM appears to be of the same order (30-57µg$CHCl_3$/mgDOC) as that of NOM extracts. It could be significantif the quantity of reactive carbon associated with the present cells and their EOM is significant. This would be the case if extreme densities of cells were chlorinated, e.g. TR bloom samples after chlorination (Figure 6.5b & d).

The absolute yield and thus the actual mass of potentially reactive NOM present in the cells is a factor to consider when evaluating whether significant competition for the free oxidant can occur. Reported DBP yields were obtained using intensive culture conditions and severe chlorination dosages that are not representative of natural blooms and treatment plant chlorination practice (Table 6.1). The carbon content of our suspension of 200,000 and 50,000 cells/mL CB were estimated from the measured biovolume of CB cells (µm^3/cell) and their dry weight (pg/cell) (Table 6.4). Optimum content of carbon (C) in CB cell is ~50% of the cell dry weight (Reynolds, 1984), corresponding to <5 mg/L of cell carbon in the suspensions. Using the highest published chloroform yield for chlorination (µgCHCl3/mgC) of *Anabaena* and *Microcystis* (Table 6.1 – data not available for other species), a maximum potential formation of <269 µg/L could be expected (Table 6.4). Actual THM yield from milder chlorination using the 2 and 5 mg/L within a contact time of 60 minutes dose did not result in these maximum THM values (Figure 6.5a & c). Figure 6.5 shows that TTHM and HAA9 yields were generally similar for Prospect water. However in TR water the HAA9 yield was much lower compared with the TTHM yield. Considering the Cl_2 residual at the end of the experiments (Figure 6.1), it is essential for operators of DWTP to deal with the possibility of further DBP formation after the pre-chlorination process.

6.5 Conclusion

This study shows that low chlorine doses resulted in the quick release and subsequent oxidation of dissolved and cell bound toxins from the CB toxic strains. Furthermore, the results showed that toxin release and subsequent oxidation occurred with complete loss of cell viability. The

detailed investigation of STXs, MC-LR and CYL release and oxidation rates indicated that toxin release rates may not always be the limiting step in the chlorination process. Kinetic analysis of the oxidation of the toxins revealed significant differences in their susceptibility to oxidation by chlorine which was dependent upon water pH. Consequently, attention should be given to provide adequate CT to guarantee the efficient oxidation of the released recalcitrant cyanotoxin analogues. These data provide novel information for DWTP to establish if the toxin release or oxidation rate is the limiting step in the toxin oxidation. However, in case of CB species producing T&O compounds, e.g. geosmin and MIB, utilities might still face the challenge of odor due to released compounds.

Results from this study suggest that pre-chlorination of natural water containing CB cell numbers ≤200,000 cells/mL for different treatment purposes is a feasible practice. The water background quality is the key factor in DBP formation potential independent of CB cell-bound material. Under the conditions employed in this study the DBP formation was below the guideline levels. These results show that it is possible to achieve conflicting water quality goals (effective toxin oxidation while respecting DBP guidelines) using chlorination conditions that would be experienced in a DWTP.

6.6 Acknowledgments

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6.8 Supplementary data (SD)

Fate of toxic cyanobacterial cells and disinfection by-products formation after chlorination

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Supplementary data (SD)

Number of pages: 7

Number of figures: 3

Number of table: 2

Table SD6-*S:* The main cyanotoxins of interest and cyanobacteria (CB) species producing these toxins (Catterall, 1980; Höger, 2003; Humpage, 2008; Merel et al., 2010; Newcombe et al., 2010; Svrcek & Smith, 2004).

Cyanotoxin	LD_{50}* (i.p. mouse µg/kg body weight)	CB species known to produce the toxins	Mechanism of toxicity	Chemical structure	Health-based exposure guidelines/recommended alert levels
Microcystins (MCs) in general (~60 known analogues) MC-LR MC-LA MC-YR MC-RR	45 - >1000 50 (25-125) 50 70 300 - 600	*Microcystis, Anabaena, Planktothrix, Oscillatoria, Nostoc, Hapalosiphon, Anabaenopsis, Aphanizomenon*	Hepatotoxic: Protein phosphatase blockers by covalent binding and cause haemorrhaging of the liver	Cyclic peptides with the amino acid ADDA	South Africa: 0.8 µg/L MC-LR World Health Organization (WHO), Czech Republic, China, France, Italy, Japan, Korea, New Zealand, Norway, Poland: 1 µg/L MC-LR Brazil, Spain: 1 µg/L MCs Australia: 1.3 µg/L MC-LR toxicity equivalent Canada: 1.5 µg/L MC-LR toxicity
Saxitoxins (STXs) also known as paralytic shellfish poisons in general (~30 known analogues) Saxitoxin (STX) C-toxin1 & 2(C1 & C2) Gonyautoxin2 & 3 (GTX2 & GTX3)	10 - 30	*Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya*	Neurotoxin: STXs are potent voltage-gated sodium channel antagonists, causing numbness, paralysis and death by respiratory arrest. STX disrupts the nervous system via binding to the sodium channel and inhibits the sodium ions transport	Carbamate alkaloids ($C_{10}H_{17}N_7O_4$) based on a 3,4,6-trialkyl tetrahydropurine skeleton which can be further carbamylated, sulphated or N-sulphocarbamylated: non-sulphated STX, singly sulphated GTX, doubly sulphated C	Australia, Brazil & New Zealand: 3.0 µg/L STX toxicity equivalent
Cylindrospermopsin (CYL)	200 - 2100	*Cylindrospermopsis, Aphanizomenon, Umezakia, Raphidiopsis, Anabaena*	Cytotoxic: blocks protein synthesis	Cyclic guanidine alkaloid	New Zealand: 1 µg/L Brazil: 15 µg/L
* LD_{50} is the dose of toxins that is lethal to 50% of the test population					

Toxin	R1	R2	R3	R5	Net Charge	Relative mouse toxicity
R4 = $CONH_2$ (carbamate toxins)						
STX	H	H	H	OH	+2	1.000
GTX2	H	H	OSO_3^-	OH	+1	0.359
GTX3	H	OSO_3^-	H	OH	+1	0.638
R4 = $CONHSO_3^-$ (n-sulfocarbamoyl (sulfamate) toxins)						
C1	H	H	OSO_3^-	OH	0	0.006
C2	H	OSO_3^-	H	OH	0	0.096

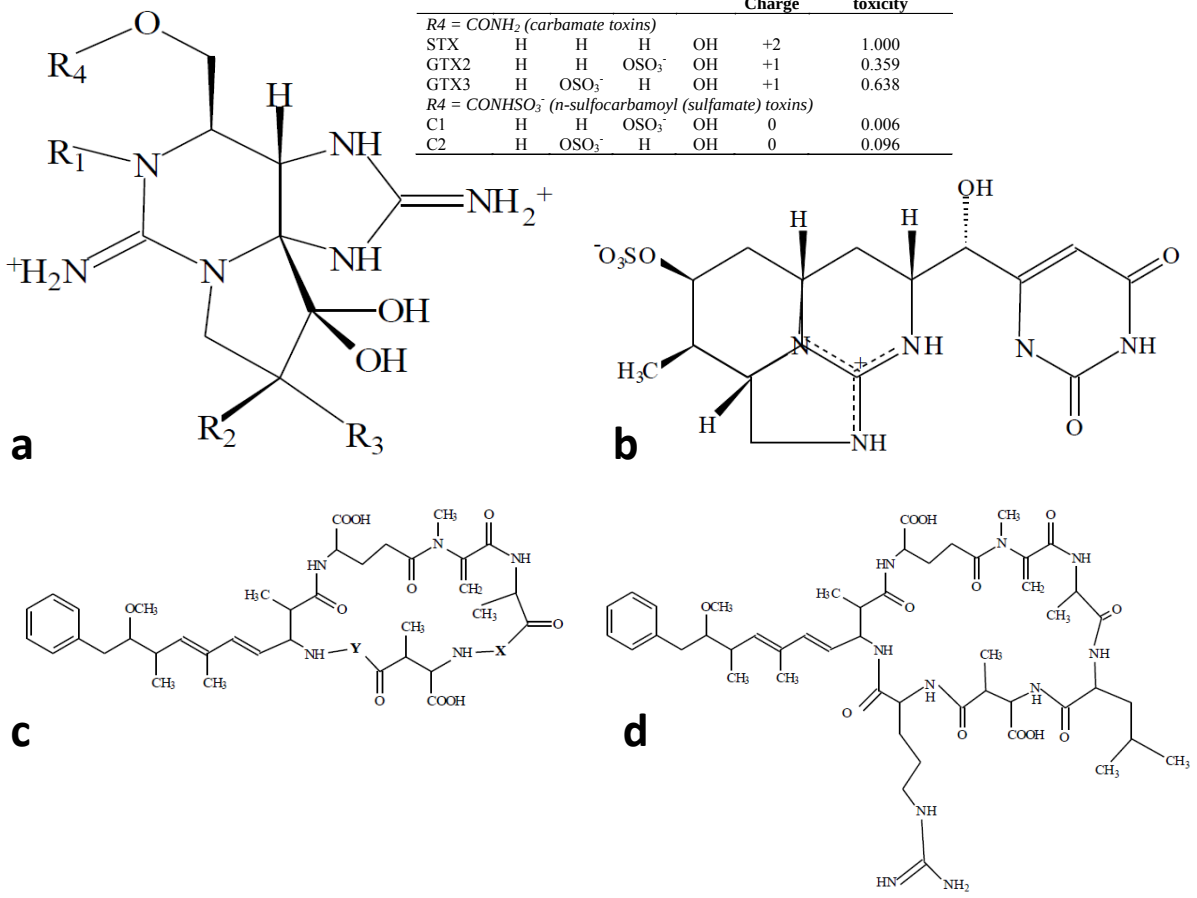

Figure SD6.*S2*: General structure of (a) Saxitoxins (STXs – also known as paralytic shellfish poison) and its mostly known analogues, (b) *cylindrospermopsin* (CYL), (c) general structure of microcystins with variant amino acids at positions X and Y, and (d) microcystin-LR (MC-LR) (Newcombe et al., 2010; Zamyadi et al., 2010).

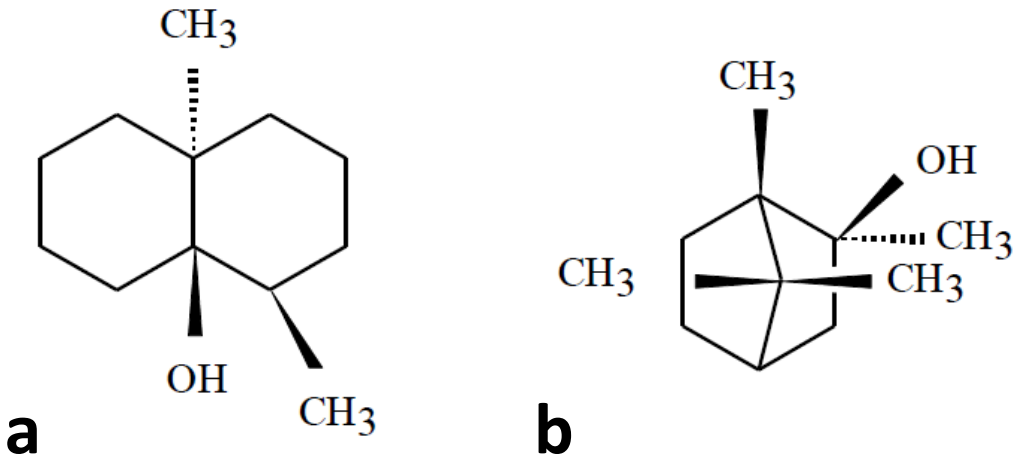

Figure SD6.*S3*: General structure of the most common CB taste and odor compounds with earthy-musty smelling (a) geosmin* and (b) 2-methylisoborneol (MIB) (Newcombe et al., 2010).

* Cell damage may cause the subsequent release of geosmin and MIB (Lin et al., 2009; Peterson et al., 1995) and chlorination is largely ineffective for geosmin oxidation (Ho et al., 2009).

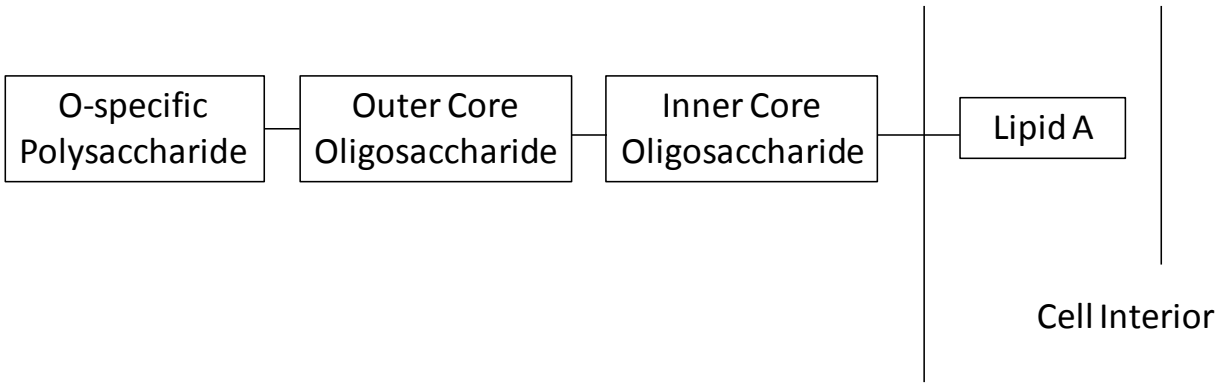

Figure SD6.*S:* General schematic of the basic lipopolysaccharides (LPS)* structure (adapted from: Stewart et al. (2006))

* Based on their overall cell structure, CB are considered to be Gram negative but their cell membranes have specific features such as a thicker peptoglycan layer and different LPS composition. Cell membranes of Gram negative bacteria such as CB are richer in LPS and phospholipids that are less reactive than the peptidoglycan cell wall of Gram positive bacteria (Hoiczyk & Hansel, 2000; Stewart et al., 2006).

Table SD6-*S5*: Validation of the accuracy of ELISA plates for STXs (µg/L of STX eq) and CYL (µg/L) analysis

Water sample	**µg/L**	**Standard deviation on triplicates**
Abraxis Saxitoxin ELISA Plate		
High purity water	0.0	0.00
High purity water + sodium thiosulphate	0.0	0.00
High purity water + 1 µg/L STX	1.0	0.04
High purity water + 1 µg/L STX + sodium thiosulphate	1.0	0.06
Beacon Cylindrospermopsin Plate kit		
High purity water	0.0	0.00
High purity water + sodium thiosulphate	0.0	0.00
High purity water + 1 µg/L CYL	1.0	0.06
High purity water + 1 µg/L CYL + sodium thiosulphate	1.0	0.05

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CHAPTER 7 ADDITIONAL RESULTS ON CHLORINATION

A draft of a paper to be submitted to a peer-reviewed journal is presented in this chapter. This draft presents the results on chlorination of toxic *M. aeruginosa* cells. This chapter studies the details of chlorine demand per cell of *M. aeruginosa*, the toxins and DOC release due to cell lysis under chlorination, oxidation of released toxins, and DBP formation potential of released cellular material. The title of this draft manuscript is:

Microcystis aeruginosa cellular chlorine demand and chlorination of released microcystin-LR in presence of cellular debris (*draft version*)

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7.1 Abstract

The presence of toxic cyanobacteria is a growing concern in drinking water sources. Direct chlorination of toxic CB cells might occur due to different treatment purposes resulting in cell lysis, toxin release and disinfection by-products (DBP) formation. There is limited information available on chlorine demand of *Microcystis aeruginosa* cells, and microcystins chlorination and DBP formation in presence of cellular materials. This research: (1) determines the extent of *M. aeruginosa* cells lysis, toxins and DOC release, and microcystins oxidation during chlorination of ultrapure and natural water spiked with laboratory cultures; (2) estimates the *M. aeruginosa* cellular chlorine demand when cell lysis occur due to chlorination; and (3) studies the DBP formation associated with the chlorination of *M. aeruginosa* cell-bound materials. Chlorine exposure (CT) values of 172.4 and 100 mg.min/L were required to obtain 76% cell lysis and oxidation of released cell-bound toxins to below guideline value of 1 µg/L MC-LR, respectively. For chlorination experiments in ultrapure water toxin oxidation rates were similar or faster than cell lysis rates. In the case of chlorination experiments in natural water, in most cases the cell lysis was faster than toxin oxidation rate. Additionally, concentrations of trihalomethanes (12.87 µg/L) and haloacetic acids (below detection limit) as DBP were below the guideline values in chlorination of 500,000 cell/mL in ultrapure water even with a maximum CT value of 3051.2 mg.min/L.

7.2 Introduction

Several toxic cyanobacteria (CB) have been increasingly detected in drinking water (DW) sources and treated water (AFSSA-AFSSET, 2006; Lahti, 2001; Zamyadi, Wang et al., 2010) Microcystins (MCs) are classified as hepatotoxins and are one of the main toxins of interest and (The Cooperative Research Centre for Water Quality and Treatment (Australia), 2007). MCs are cyclic peptides with the amino acid ADDA with over 60 known analogous (Fischer et al., 2001; Merel et al., 2009; Svrcek & Smith, 2004). The more commonly found analogues are microcystin-LR (MC-LR), MC-LA, MC-YR, and MC-RR. *Microcystis aeruginosa* is one the main CB species producing these hepatotoxins and freshwater *M. aeruginosa* proliferation events have been observed across the globe (Svrcek & Smith, 2004). MCs caused discontinuous but repeated extensive poisoning of wild and domestic animals, fish and humans (Svrcek & Smith, 2004).

Acute toxicity essays demonstrated that the dosages of different MCs analogous lethal to 50% of the test population (LD_{50}) vary markedly ranging from 50 to >1200 mouse µg/kg body weight. Acute hepatotoxicosis, caused by hepatotoxins, is the most commonly encountered toxicity involving CB. The acute mode of hepatotoxic action is to cause cell–cell separations in the liver cells (hepatocytes), allowing for accumulation of blood in the liver and eventual death of the exposed living being by hemorrhagic (internal bleeding) shock or liver failure. The cyclic peptides have been shown to produce liver tumors in laboratory rodents, and there is also indirect evidence that MCs may promote tumors in humans from DW. MCs toxicity depends on the degree of methylation of MeAsp and Mdha amino acids, and on the stereoisomers of the Adda chain (Svrcek and Smith 2004). Consequently the concentration of MCs in DW is regulated by different water authorities across the globe (Newcombe et al., 2010) including World Health Organization (WHO) guideline limit of 1 µg/L of MC-LR for safe DW (Chorus & Bartram, 1999). WHO recommends (Chorus & Bartram, 1999) that in water containing MCs producing CB, the MC-LR guideline value should be applied to the total cell-bound and extracellular MCs.

It is suggested that global climate change may have profound effects on runoff, watershed phosphorus load, ecological state of water bodies and water temperature (Jeppesen, 2009). These effects enhance the dominance of potentially toxic CB, including toxic *M. aeruginosa*, and their intense proliferation in drinking water (DW) sources (Dale, 2006; Elliott et al., 2006; Jöhnk et

al., 2008; Paul, 2008). The health relevance of *M. aeruginosa* and their associated toxins, the treatment challenges to remove these compounds and the observed trends of growing predominance of CB in surface water raise the issue of their presence in water sources that were not previously considered at high risk of cyanotoxin contamination. Recent preventive monitoring of CB in several drinking water treatment plants (DWTPs) in Quebec (Canada) has evidenced the breakthrough and accumulation of toxic CB, with dominance of MCs producing *M. aeruginosa*. Furthermore, the observation of toxins release during the conventional treatment process suggests that the post-oxidation, mainly chlorination in these systems, is the final barrier against the cyanotoxins before production of DW (McQuaid et al., 2011; Zamyadi, Wang et al., 2010). It has been discussed previously that direct chlorination of raw water is used for different treatment purposes and if conducted on CB containing water it will cause serious water quality concerns, e.g. cell damage and the subsequent release of cell-bound toxins, increased chlorine demand, dissolved organic carbon (DOC) release and subsequent chlorination by-products (DBP) formation (Merel et al., 2010; Zamyadi, Ho et al., 2010). There is a need to understand the limitation of pre- and post chlorination of *M. aeruginosa* cells and their associated toxins as used during DWTP operation.

Chlorination has been shown to be efficient in oxidation of *M. aeruginosa* and MCs (Daly et al., 2007; Merel et al., 2010). The chlorine doses required to maintain a residual in the distribution system (0.5 mg/L after 30 minutes of contact time) at the moderate water temperatures (~20^{o}C) with pH values under 8, results in oxidation of MCs below detection limits (Newcombe& Nicholson, 2004). Lin et al. (2009) and Daly et al. (2007) studied the fate of *M. aeruginosa* and their associated MCs during chlorination in natural water from Australian and Taiwan natural water samples. It is essential to verify their findings in local natural water for operational purposes. Furthermore, secure adjustment of chlorination operation in DWTP requires the knowledge of *M. aeruginosa* cellular chlorine demand, which is currently unknown.

The chlorine concentration and the contact time are the key factors in confident application of chlorine for oxidation purposes. The appropriate degree of oxidation is obtained using the concept of concentration multiplied by time, chlorine exposure, or CT. The CT value is calculated by determining the area under a graph of chlorine concentration versus time (Ho et al., 2006). The degree of cell-bound and extracellular MCs chlorination can be predicted by

quantifying the rates of cell lysis, MCs release, and MCs oxidation under given water quality conditions (Daly et al., 2007).

It has been suggested that the chlorine demand of CB cell-bound compounds and the consequent DBP formation potentials varies (Bond et al., 2009). Release of cell-bound compounds, due to autolysis of cells classified as extracellular organic matter (EOM) and/or due to compromised lysis categorised as internal organic matter (IOM) (Fang, 2010) could contribute to the formation of DBPs, e.g. trihalomethanes (THM) and haloacetic acids (HAA), due to reactivity of released material with chlorine (Bond et al., 2009; Hureiki et al., 1994). Published results on the DBP formation potentials of IOM and EOM from cellular suspensions under chlorination are contradictory. Significant DBP formation has been reported in connection to reactive carbon associated with high cell numbers and their EOM (Graham, 1998; Huang et al., 2009). However, chlorination of approximately 46,000 cells/mL of *Anabaena circinalis* cells in natural water (complete cell lyses/damage and below detection limit oxidation of produced saxitoxins) with a CT of 50.3 mg.min/L caused an increase of 9 µg/L of total THM (TTHM) and 3 µg/L of HAA9 compared to the chlorination of control natural water without CB cells (Zamyadi, Ho et al., 2010).

Systematic studies are needed to better understand the chlorine demand per cell of *M. aeruginosa* and DBP formation due to chlorine contact with cell materials in conditions close to DWTP operational situation. The objectives of this research were: (1) to determine the extent of *M. aeruginosa* cells lysis, toxins and DOC release, and MCs oxidation during chlorination of ultrapure and natural water spiked with laboratory cultures; (2) to estimate the *M. aeruginosa* cellular chlorine demand when cell lysis occur due to chlorination; and (3) to study the DBP formation associated with the chlorination of *M. aeruginosa* cell-bound materials. To the best of our knowledge this paper presents the first quantitative study of estimation of *M. aeruginosa* cellular chlorine demand and DBP formation under chlorination.

7.3 Materials and Methods

7.3.1 Materials/Chemicals and Reagents

A toxic strain of *M. aeruginosa*, isolated from Pretzlaff Pond, Alberta, Canada, was cultured in the laboratory in artificial seawater medium (ASM-1) according to the method of Gorham et al.

(1964). Distilled water (20 L) was fortified with nutrients according to the ASM-1 preparation, sterilized at 120^{o}C, and then after pH adjustment, inoculated with an ASM-1 grown culture in stationary phase. The culture was incubated at 26^{o}C under 6 hours rotating light-darkness flux at light intensity of 70 µmol s^{-1} m^{-2}. All chemicals and reagents used for analytical methods calibration were laboratory analytical grade from various suppliers.

7.3.2 Water Source and Water Quality Characterisation

Water was sourced from the Mille-Il River (MIR) in Quebec, Canada. Samples were collected from the St-Rose DWTP intake prior to any treatment. Untreated MIR water was filtered over pre-rinsed Binder-free glass microfiber 1.2µm filter (GF/C - Whatman, UK) prior to inoculation with culture. The water had a DOC concentration of 6.3 mg/L, UV absorbance (at 254 nm) of 0.191 cm^{-1}, pH of 7.4, and an alkalinity of 19 mg/L as $CaCO_3$. Ultrapure water (Millipore Pty Ltd, USA) was also used for selected experiments. Prior to DOC analyses, samples were passed through pre-rinsed (1 L ultrapure water) 0.45 µm cellulose nitrate Supor®-450 membrane filters (PALL Life Sciences, USA). DOC measurements were made on a 5310C total organic carbon analyzer (Sievers Instruments Inc., USA). pH was measured on a Fisher Scientific pH meter (Accumet, Fisher Scientific Instruments, USA) which was calibrated with pH 4, 7 and 10 standard buffers (Fisher, USA).

7.3.3 Chlorination experiments

Liquid chlorine stock solution was purchased from Fisher (USA). Chlorine stock and chlorine residual concentrations were determined using the DPD-FAS titration method described in Standard Methods (American Public Health Association (APHA) and American Water Works Association (AWWA), 2005). Typical chlorine stock solutions concentration is 50,000 mg/L as free chlorine. Three separate chlorination experiments were conducted on (1st Cl_2 Exp) MCs producing *M. aeruginosa* suspensions in ultrapure water, (2nd Cl_2 Exp) MCs producing *M. aeruginosa* suspensions in MIR water, and (3rd Cl_2 Exp) cell-bound *M. aeruginosa* materials. Each chlorination experiment was presided by a chlorine decay experiment. For chlorine decay experiments, water samples were dosed with desired chlorine concentration and triplicate samples taken at specified contact times for free chlorine residual determinations using the

titration method. All chlorination experiments were conducted in triplicate in brown amber bottles at room temperature (20 ± 2^{o}C).

Cultured *M. aeruginosa* cells were enumerated on a compound microscope in a Sedgewick-Rafter counting chamber after preservation in Lugol‟s iodine (Cooperative Research Centre for Freshwater Ecology (CRCFE); Australia, 1999). Required volume of stationary phase culture to be spiked in ultrapure and MIR water to achieve desired cell suspension was adjusted based on the culture cell number. The number of cells in water samples after inoculation was confirmed by microscopic enumeration. Control (non-chlorinated) water samples showed no sign of cell integrity loss due to direct addition of cells to ultrapure and MIR water (data not shown) similar to data from Zamyadi, Ho et al. (2010).

1st Cl_2 Exp: These experiments were conducted in ultrapure water with controlled pH 8 by the addition of phosphate buffer (0.01 M) (APHA-AWWA, 2005). Chlorine was added from the chlorine stock solution to obtain the Cl_2 doses of 2, 3 and 4.5 mg/L for *M. aeruginosa* suspensions of 60,000, 250,000 and 500,000 cells/mL, respectively. Samples were quenched at specified contact times with sodium thiosulphate at a stoichiometric ratio specified in Standard Methods (APHA-AWWA, 2005). Quenched samples were taken for DOC measurement. To determine the cell lysis due to chlorination total cell counts (microscopic counts in triplicate) were conducted on 2 mL samples after preservation in Lugol‟s iodine (CRCFE—Australia, 1999). Remaining quenched water samples were divided in two subsamples of 250 mL: the first subsample was immediately filtered through 0.45 µm filter (Whatman, UK) to remove cellular material, and then analyzed for extracellular MCs. The second subsample was subjected to three sonication cycles before filtration over 0.45 µm filter to analyze for total MCs (both the cell-bound and extracellular compounds). For all MCs analysis, confidence limits were calculated using the standard deviations of triplicate measurements. For all experiments the extracellular concentrations of MCs is always fitted in the confidence limit of total concentration. The difference between total and extracellular levels of MCs was estimated as cell-bound concentration.

2nd Cl_2 Exp: These experiments were conducted in MIR water with controlled pH values of 7 and 8.5 by addition of the similar buffer. Chlorine was added from the chlorine stock solution to obtain the Cl_2 doses of 2, 5 and 10 mg/L for *M. aeruginosa* suspensions of 50,000 cells/mL.

Samples were quenched at specified contact times with sodium thiosulphate at a stoichiometric ratio (specified in Standard Methods). Quenched samples were taken for DOC measurement. To determine the cell lysis due to chlorination total cell counts (microscopic counts in triplicate) were conducted on 2 mL samples after preservation in Lugol‟s iodine. A volume of 250 mL from remaining quenched water samples was subjected to three sonication cycles before filtration over 0.45 μm filter (Whatman, UK) to analyze for total MCs.

3rd Cl_2 Exp: These experiments were conducted on *M. aeruginosa* suspension of 500,000 cells/mL in ultrapure water with controlled pH 8 (similar buffer method). The *M. aeruginosa* suspension was subjected to three sonication cycles for complete release of intracellular material before chlorination. Chlorine was added from the chlorine stock solution to obtain the Cl_2 dose of 5.5 mg/L. Samples were quenched after 30 and 1440 minutes of contact times at a stoichiometric ratio (specified in Standard Methods). Quenched samples were taken for DOC and DBP measurements.

7.3.4 Analysis of MCs and DBP

MCs analyses were conducted using an Abraxis Microcystins ADDA ELISA Plate (an enzyme-linked immunosorbent assay – Abraxis LLC, Pennsylvania, USA). The analyses of MCs concentrations were measured as μg/L of MC-LR equivalent (MC-LR eq.). The accuracy of the Plate was validated using standard toxin materials (data not shown). DBP analyses were conducted by the laboratory of NSERC Industrial Chair on Drinking Water at Ecole Polytechnique de Montreal (Canada) using the method described by Koch et al. (1988) for THM analysis and Hodgeson et al. (1995) for HAA. Chlorinated water samples from the 3rd Cl_2 Exp were analyzed for four THM components (bromoform, chloroform, bromodichloromethane, and dibromochloromethane) as total THM (TTHM) and six HAA compounds (Bromoacetic acid, Bromochloroacetic acid, Chloroacetic acid, Dibromoacetic acid, Dichloroacetic acid, and Trichloroacetic acid) as HAA6.

7.3.5 Data analysis

It has been observed that during the chlorination experiment the reaction between chlorine and the *M. aeruginosa* cells or MCs is of second order (Daly et al., 2007; Lin et al., 2009). Equation

1 was used to calculate the chlorination second-order apparent rate constants for these chlorination experiments (Daly et al., 2007; Lin et al., 2009):

$$Ln\left(\frac{[X_{CT}]}{[X_0]}\right) = -k \times CT \qquad \text{Equation 1}$$

where CT equals the chlorine exposure; X_{CT} equals the number of lysed cells or MC-LR eq. concentration after a given chlorine exposure; X_0 equals the number lysed cells or MC-LR eq. concentration at CT=0; and *k* equals the rate at which *M. aeruginosa* cells (k_{lysis}) or MC-LR eq (k_{appt}) oxidation occurs (Daly et al. 2007; Lin et al. 2009).

7.4 Results and discussion

7.4.1 Impact of M. aeruginosa cellular compounds and water matrix on chlorine demand

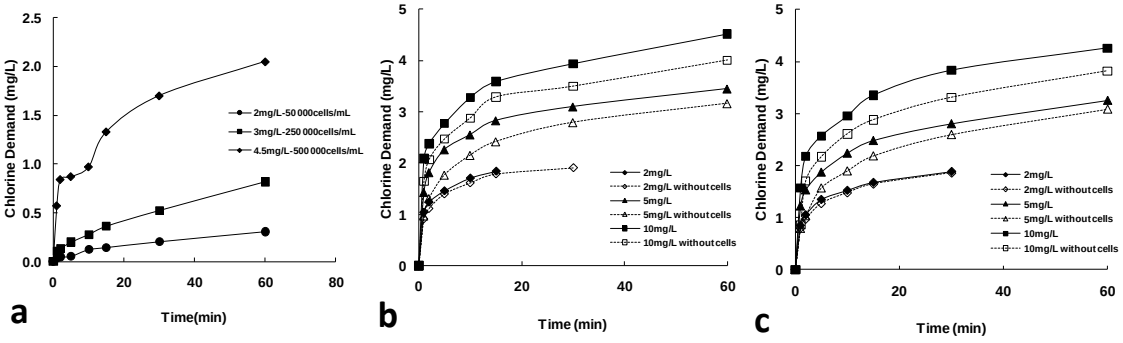

Figure 7.1: Chlorine demand of *M. aeruginosa* suspensions in (a) ultrapure water with 60,000, 250,000 and 500,000 cells/mL under 2, 3 and 4.5 mg/L Cl_2 respectively at pH 8, and 50,000 cell/mL under 2, 5 and 10 mg/L Cl_2 (b) at pH 7 and (c) pH 8.5.

Free chlorine decay and cell lysis experiments were conducted using the *M. aeruginosa* culture spiked in ultrapure (60,000±2%, 250,000±4% and 500,000±4% cells/mL with 2, 3 and 4.5 mg/L of Cl_2 at pH 8) and MIR (50,000±5% cells/mL with 2, 5 and 10 mg/L of Cl_2 at pH 7 and 8.5) water (Figure 7.1). The standard deviation value of these cell enumerations (triplicate enumeration/sample) fit within the confidence limit of cell count method (Hurst et al., 2007;

Laslett et al., 1997; Lund et al., 1958) (data not shown). Chlorine demand in ultrapure water was directly related to the *M. aeruginosa* cell numbers (Figure 7.1a). The chlorine demand in MIR water in the absence of cells was lower than in the presence of cells with similar trends for both pH values (Figures 7.1b & 7.1c). Chlorine decay data were used to calculate the CT values by fitting a two pathway integration model to a two phase decay curve: fast (<1 min) and slow (Daly, Ho et al., 2007; Zamyadi, Ho et al. 2010). Table 7.1 includes the resulting first order rate constants for chlorine decay. The apparent rate constants (k_{Cl2}) in ultrapure water without cells were very stable (Table 7.1). The addition of CB cell to the ultrapure water augments the apparent rate constant values (Table 7.1). Also, the k_{Cl2} values increased with the increase in cell numbers and chlorine doses. Increase in cell number caused higher DOC values (60,000, 250,000 and 500,000 cells/mL corresponds to 0.17, 0.75 and 1.08 mg/L of DOC) and consequently higher k_{Cl2} values were observed.

In MIR water the apparent rate constants were similar under the two pH values (Table 7.1). The apparent rate constant values increase with the addition of cells to MIR water and the increase of chlorine doses (Table 7.1). The cell number (50,000 cells/mL corresponding to 0.12 mg/L DOC) and MIR water DOC were constant as chlorine concentration increases. All the chlorination experiments regardless of water quality parameters were conducted with chlorine surplus after 60 minutes of contact.

Table 7.1: First-order apparent rate constants (k_{Cl2}) in s^{-1} for chlorine decay. All correlation coefficients (R^2) exceeded 0.85.

Water	Cell nb. Cells/mL	2 mg/L Cl_2		3 mg/L Cl_2		4.5 mg/L Cl_2	
		pH 8		pH 8		pH 8	
Ultrapure	No cell	1.7×10^{-5}		1.6×10^{-5}		1.7×10^{-5}	
	60,000	6.4×10^{-5}		-		-	
	250,000	-		9.7×10^{-5}		-	
	500,000	-		-		1.5×10^{-4}	
Water	Cell nb. Cells/mL	2 mg/L Cl_2		5 mg/L Cl_2		10 mg/L Cl_2	
		pH 7	pH 8.5	pH 7	pH 8.5	pH 7	pH 8.5
MIR	No cell	8.4×10^{-5}	8.1×10^{-5}	1.8×10^{-4}	1.8×10^{-4}	1.1×10^{-3}	1.1×10^{-3}
	50,000	8.4×10^{-5}	8.2×10^{-5}	1.9×10^{-4}	1.9×10^{-4}	1.9×10^{-3}	1.9×10^{-3}

7.4.2 Chlorination effect on *M. aeruginosa* cells lysis and estimation of chlorine demand per cell

The total cell count was used to assess the impact of chlorination on reduction of cell numbers. In this study cell lysis is defined as the status of comprised cell that is undetectable under the microscope in preserved sample. At first, the response of three *M. aeruginosa* cell suspensions of 60,000±2%, 250,000±4% and 500,000±4% cells/mL in ultrapure water (pH 8) to respectively 2, 3 and 4.5 mg/L of chlorine was evaluated. Figure 7.2a demonstrate that chlorination in ultrapure water with highest cell number caused 76% of cell lysis after 60 minutes of contact (CT = 172.43 mg.min/L). Additionally, the response of the *M. aeruginosa* cell suspensions of 50,000±5% cells/mL in MIR water (pH 7 and 8.5) to 2, 5 and 10 mg/L of Cl_2 was studied. Figure 7.2b shows that similar cell lysis was achieved with the same or higher CT values in MIR water. Zamyadi, Ho et al. (2010) observed 15% of reduction in total cell numbers (lysis) with a CT of ~50 mg.min/L during chlorination of approximately 46,000 cell/mL of *Anabaena circinalis*. However, they have noted (Zamyadi, Ho et al., 2010) that over 98% of cells (even detectable under microscope in preserved samples) were categorized as injured/dead cells (damaged membrane or metabolically inactive cells) with a chlorination CT of less than 7 mg.min/L.

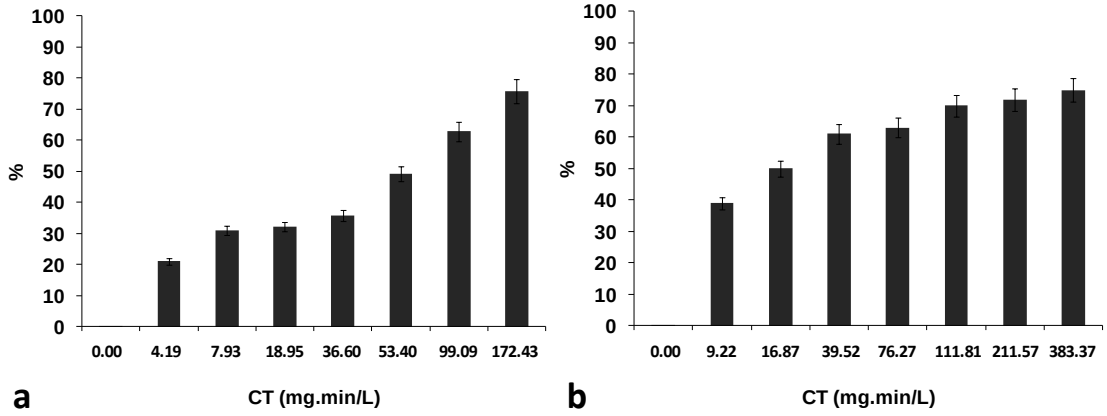

Figure 7.2: Reduction in number of cells (cell lysis) during chlorination of *M. aeruginosa* suspensions of (a) 500,000 cells/mL in ultrapure water at pH 8 with 4.5 mg/L Cl_2 (similar results were observed in other chlorination experiments in ultrapure water) and (b) 50,000 cells/mL in MIR water at pH 8.5 with 10 mg/L Cl_2 (similar results were observed in other chlorination experiments in MIR water).

Assumption of a first-order reaction with respect to chlorine reaction and the *M. aeruginosa* cells lysis is feasible (Daly et al., 2007; Lin et al., 2009). Hence the reaction between chlorine and the cells is of second-order and the constant rate at which cell lysis occurs (k_{lysis}) is evaluated using equation 1. The k_{lysis} calculated for the *M. aeruginosa* chlorination experiments in this study are presented in Table 7.2. Lower k_{lysis} values were observed in ultrapure water compared to the natural water. However, chlorine per cell numbers ratio in ultrapure water experiments were lower than in MIR water. The k_{lysis} values in MIR water are within or close to chlorination rates estimated by Ding et al. (2010) for laboratory cultures of *M. aeruginosa* and Lin et al. (2009) in natural bloom samples (Table 7.2). Furthermore in other studies cell lysis included cell membrane damage and reduction in total cell numbers, while in this study only the final phase of reduction of total cell numbers is included in estimation of cell lysis rate. The physiological state of the *M. aeruginosa* cells, cell densities, water matrix and the chlorination conditions manipulate the vulnerability of *M. aeruginosa* cells to chlorination (Pietsch et al., 2002; Lin et al., 2009).

The chlorination of *M. aeruginosa* suspensions in ultrapure water in presence of chlorine residual (Figure 7.1) and the count of lysed cells due to chlorination (Figure 7.2) permits to estimate the *M. aeruginosa* cellular chlorine demand. The accumulative chlorine demand after 60 minutes of contact per number of lysed cells is the constant value of 5.6±0.2 $pgCl_2$/cell for the three experiments conducted in ultrapure water. This novel information on cellular chlorine demand will help the operators of the DWTPs to estimate if their chlorination practice satisfies the total chlorine demand of CB containing waters based on cell count results.

7.4.3 Chlorination effect on cell-bound and extracellular MCs release and oxidation

Figure 7.3 shows the release and oxidation of cell-bound MCs (MC-LR eq.) during chlorination experiments conducted in ultrapure water. For the three *M. aeruginosa* suspensions of 60,000, 250,000 and 500,000 cells/mL complete toxin release (overlap of the total toxins line and the dissolved toxins line) and below the WHO guideline value (1 µg/L MC-LR for DW) oxidation of the toxins is observed with a CT of 100 mg.min/L (Figure 7.3). Figure 7.4 demonstrates the oxidation of total MC-LR eq. from 50,000 cells/mL in MIR water at pH 7 (Figure 7.4a) and pH 8.5 (Figure 7.4b) during chlorination with three Cl_2 doses (2, 5, and 10 mg/L Cl_2). Chlorination with 2 mg/L of Cl_2 caused over 80% removal of the toxins (Figure 7.4). In the case of 5 and 10

mg/L of Cl_2 a CT value of 100 mg.min/L was sufficient for below detection limit oxidation of the total MC-LR eq. (Figure 7.4). The chlorination at pH 7 and pH 8.5 resulted in similar outcomes. The observed toxins release and oxidation (Figure 7.3 and 7.4) are in good coherence with the reduction of cell numbers (cell lysis) due to chlorination (Figure 7.2).

Previous publications (Daly et al., 2007; Lin et al., 2009; Rodriguez et al., 2007; Zamyadi, Ho et al., 2010) demonstrated the application of kinetics models to express the chlorination of released cell-bound and dissolved MCs. The kinetics models were defined by the apparent rate constant for the toxin chlorination and the chlorine decay in the water. A first-order reaction is supposed with respect to chlorine and the concentration of the MCs. Thus the reaction between chlorine and MCs is of second-order. Equation 1 uses the CT values and the rate constants to calculate the toxin concentration at a given chlorination condition. It was possible to define a second-order rate constant (k_{total}) for the oxidation of total MC-LR eq. in ultrapure and MIR water. The k_{total} is a combined rate for the simultaneous release of cell-bound toxins due to cell lysis and the oxidation of the released toxins. However in chlorination of 500,000 cells/mL of *M. aeruginosa* is ultrapure water (Figure 7.3c) enough data points were available after complete release of the cell-bound toxins to calculate the rate constant ($k_{released}$) for the chlorination of the dissolved toxins. $k_{released}$ model the oxidation of dissolved toxin in presence of cellular debris which is similar to real world conditions. The derived apparent rate constant from this study and previously published rate values are listed in Table 7.2.

Table 7.2: The apparent rate constants for cell lysis or reduction in total cell count (k_{lysis} - $M^{-1}s^{-1}$) , the apparent rate constants for total toxins oxidation (k_{total} - $M^{-1}s^{-1}$) and released cell-bound toxins oxidation ($k_{released}$ - $M^{-1}s^{-1}$) derived from this study compared to published results. Comments in parenthesis describe the origin of the CB cells (from laboratory cultures or environmental bloom samples) and the toxins (from standard extracts or cell-bound).

This study	60,000 cells/mL, Ultrapure water, 2 mg/L Cl_2, pH 8	250,000 cells/mL, Ultrapure water, 3 mg/L Cl_2, pH 8	500,000 cells/mL, Ultrapure water, 4.5 mg/L Cl_2, pH 8		50,000 cells/mL, MIR water, 2 mg/L Cl_2, pH 7	50,000 cells/mL, MIR water, 2 mg/L Cl_2, pH 8.5	50,000 cells/mL, MIR water, 5 mg/L Cl_2, pH 7	50,000 cells/mL, MIR water, 5 mg/L Cl_2, pH 8.5	50,000 cells/mL, MIR water, 10 mg/L Cl_2, pH 7	50,000 cells/mL, MIR water, 10 mg/L Cl_2, pH 8.5	Acero et al., 2005. pH 7.9-8	Ho et al., 2006. pH 7.9	Rodriguez, Onstad et al., 2007; Rodriguez, Sordo et al.,2007. pH 8	Daly et al., 2007. pH 6.8-7.6	Lin et al., 2009.,pH 8.3-8.6	Ding et al., 2010. pH 7.6
Species	$k_{lysis\ (reduction\ in\ total\ cell\ count)}$ ($M^{-1}s^{-1}$)										k_{lysis} ($M^{-1}s^{-1}$)					
M. aeruginosa	27.90	17.85	8.58		234.07	170.21	73.13	33.30	30.64	11.50	-	-	-	670±77	790-1100 (culture), 70-590 (bloom)	43.6
Toxin	k_{total}	k_{total}	k_{total}	$k_{released}$	k_{total}	k_{total}	k_{total}	k_{total}	k_{total}	k_{total}	k_{total}					
MC-LR	26.52	33.05	86.86	14.68	193.81	141.20	186.34	105.06	20.77	18.20	33.1-38.0	110-170	33 (extract)	242 (extract), 10-96 (cell-bound)	-	55.9 (dissolved)

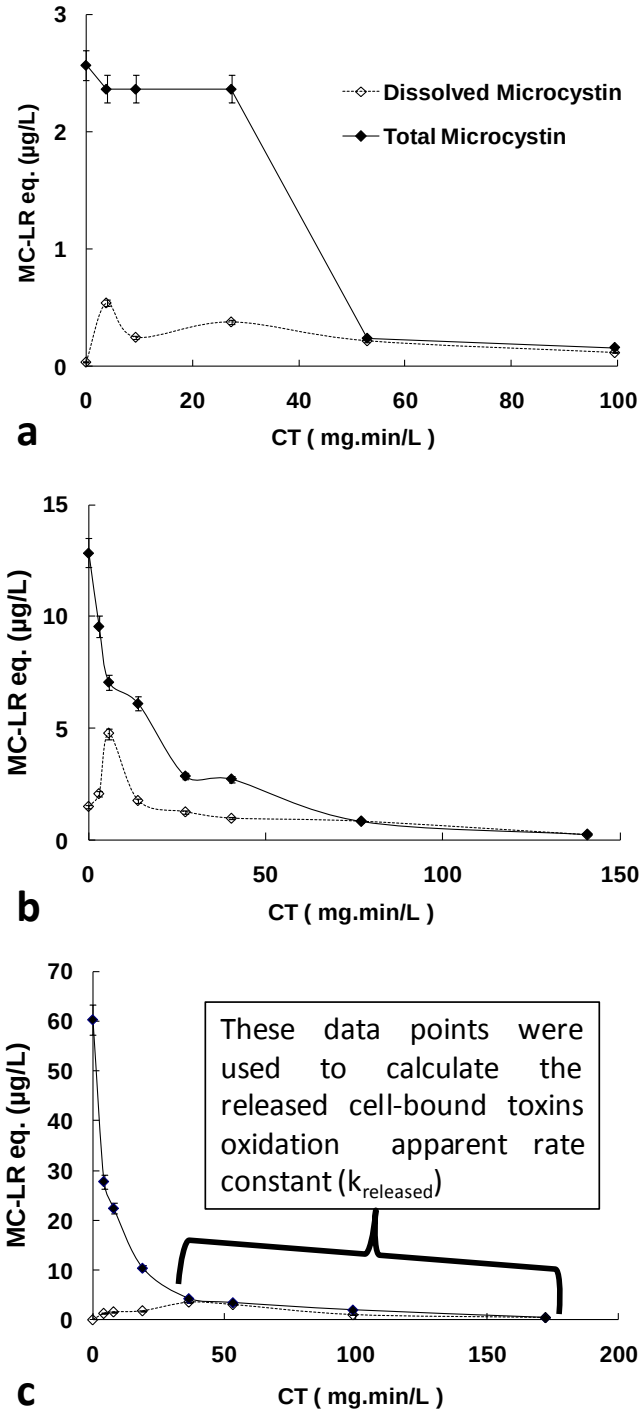

Figure 7.3: Toxins release and oxidation from *M. aeruginosa* suspensions in ultrapure water at pH 8 with cell number and chlorine doses of (a) 60,000 cells/mL and 2 mg/L Cl_2, (b) 250,000 cell/mL and 3 mg/L Cl_2, and (c) 500,000 cells/mL and 4.5 mg/L Cl_2, respectively.

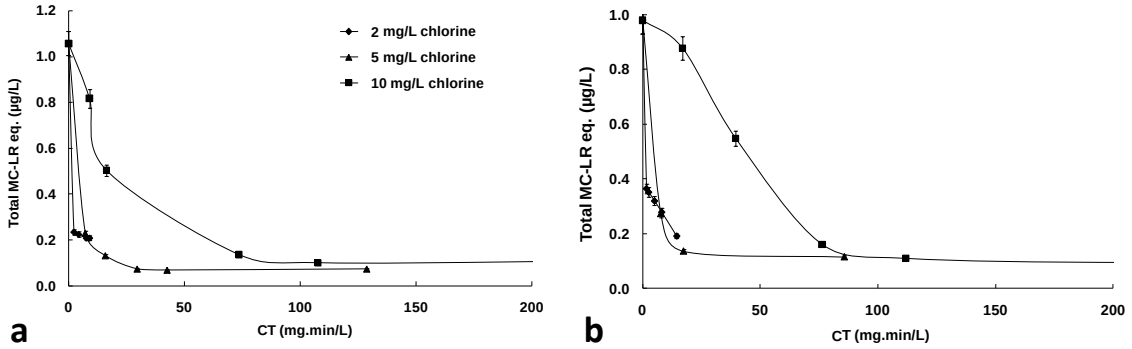

Figure 7.4: Total MC-LR eq. oxidation from *M. aeruginosa* suspensions of 50,000 cells/mL in MIR water at (a) pH 7, and (b) pH 8.5.

All chlorination essays in ultrapure and MIR water were conducted in chlorine surplus. The chlorination conditions are considered representative of operational practice in DWTPs. $k_{released}$ is the lowest MC-LR eq. chlorination rate in this study (Table 7.2) and is coherent with the MCs chlorination rate constants from Daly et al. (2007) experiment using cell-bound toxins. It was observed that oxidation of MC-LR was more efficient in chlorination experiments conducted at pH values below 8 (Ho et al., 2006). This phenomenon has been contributed to the degree of MC-LR protonation at lower pH values making it more susceptible to chlorination (Ho et al., 2006). Similar results have been obtained in this study with lower k_{total} estimated at pH 8.5 compared to pH 7 (Table 7.2).

In previous studies, similar (Ding et al., 2010) or faster (Daly et al., 2007) cell membrane damage than oxidation of the MC-LR were observed (Table 7.2). In this study for chlorination experiments in ultrapure water, similar or faster toxin oxidation rates than cell lysis rates were observed. These results highlights, the oxidation of cell-bound toxins upon release from lysed cells. In the case of chlorination experiments in MIR water, in most cases the cell lysis is faster than toxin oxidation rate. However, in two cases the toxin oxidation rate is faster than cell lysis. Ding et al. (2010) observed faster MC-LW, MC-LF, MC-RR, MC-YR and MC-LA chlorination rates (3320, 204, 136, 94 and 89.5 $M^{-1}s^{-1}$ respectively) than cell inactivation rate (43.6 $M^{-1}s^{-1}$).

These observations can be contributed to the measurement of the final cell lysis (reduction in total cell numbers) during chlorination in this study rather than gradual cell membrane damage and loss of cell viability as previously observed by Zamyadi, Ho et al. (2010).

7.4.4 Chlorination effect on cellular organic carbon release and BDP formation

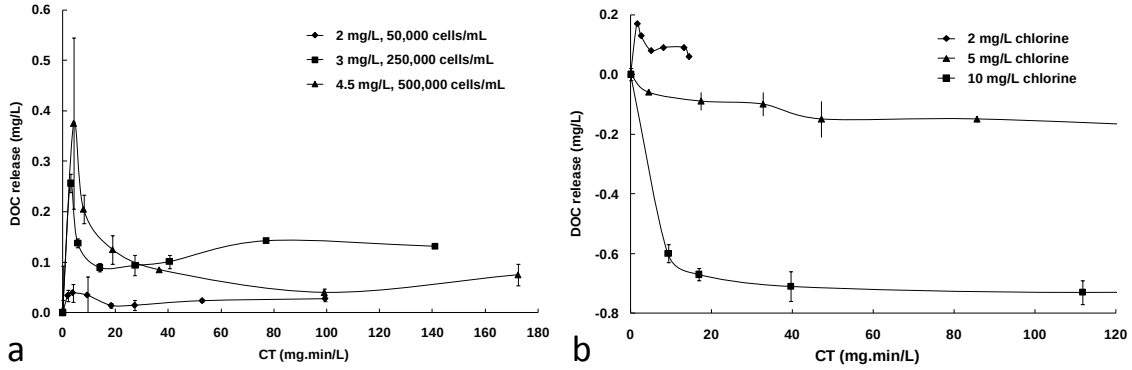

Figure 7.5: DOC release from oxidation of *M. aeruginosa* suspensions in (a) ultrapure water at pH 8 and (b) MIR water at pH 8.7 (results in MIR water at pH 7 were similar to those of pH 8.5).

Figure 7.5 demonstrates the effect of chlorination on DOC variations in *M. aeruginosa* suspensions in ultrapure and MIR water. The final DOC of cellular suspensions in ultrapure water increased after 60 minutes of chlorination compared to their DOC prior to chlorination (Figure 7.5a). However, for these experiments a maximum DOC release was observed within the first quenching time (1 minutes with a maximum CT of 4.2 mg.min/L for chlorination of 500,000 cells/mL with 4.5 mg/L of Cl_2) followed by a decrease in DOC concentration (Figure 7.5a). DOC variations during chlorination of *M. aeruginosa* suspension (50,000 cells/mL) in MIR water with 2 mg/L of Cl_2 was similar to ultrapure experiments (Figure 7.5b). Though, the chlorination of cellular suspensions (50,000 cells/mL) in MIR water with 5 and 10 mg/L of Cl_2 resulted in reduction of final DOC concentrations (Figure 7.5b). The impact of chlorine on DOC of CB cell suspensions requires further investigation. Certain DBP yields constitute CB cell-bound DOC as a significant source of DBP precursors (Huang et al., 2009; Nguyen et al., 2005; Plummer & Edzwald, 2001; Wardlaw, 1991).

One of the main objectives of this study was to study the DBP formation associated with the chlorination of EOM and IOM associated with *M. aeruginosa*. The TTHM and HAA6 formation associated with the chlorination of *M. aeruginosa* suspensions (60,000, 250,000 and 500,000 cells/mL) in ultrapure water were below detection limit (data not shown). The *M. aeruginosa* cells used for this study were harvested at stationary phase of growth. It has been suggested that the EOM release varies broadly with the algal cell growth phase and can attain intense values of 40 mg/L in extremely dense suspensions (Hoyer et al., 1985; Nguyen et al., 2005). During the exponential growth phase of CB cells low molecular weight intermediate products (e.g. glycolic and amino acids) with high reactivity with chlorine are dominant in EOM, while in aged cells (late stationary phase) high molecular weight products (e.g. polysaccharides) are abounded (Huang et al., 2009; Nguyen et al., 2005).

Huang et al. (2009) reported yield of high concentrations of THM and HAA precursors from high density *Microcystis* sp. culture (high DOC) during chlorination experiments at pH 7 with over 0.5 mg/L Cl_2 residual from 1 to 7 days of contact. To assess the impact of *M. aeruginosa* cellular IOM and EOM on DBP formation during chlorination, a suspension of 500,000 cells/mL was sonicated 3 times prior to chlorination with 5.5 mg/L Cl_2 at pH 8. Table 7.3 presents the TTHM and HAA5 resulted from chlorination of sonicated *M. aeruginosa* suspension after 1440 minutes (24 hours). The measured concentrations of TTHM and HAA5 with the CT of 3051.15 mg.min/L (after 24 hours of contact time – Table 7.3) are meeting United States Environmental Protection Agency (USEAP) regulations (80 µg/L for TTHM and 60 µg/L for HAA5) (United States Environmental Protection Agency (USEPA), 2006).

Table 7.3: DBP formation in chlorination of lysed *M. aeruginosa* suspension (sonicated with DOC of 1.30) of 500,000 cells/mL in ultrapure water at pH 8.

Time	Cl_2 residual	Cl_2 demand	CT	TTHM	HAA6
min	mg/L	mg/L	mg.min/L	µg/L	µg/L
0	5.5	-	0.00	BDL*	BDL
30	2.9	2.6	125.4	BDL	BDL
1440	1.3	4.2	3051.2	12.87	BDL

* Below Detection Limits (BDL)

The estimated biovolume of *M. aeruginosa* is 53 µm ^{3}/cell and its maximum estimated dry weight is 44 pg/cell (Reynolds, 1984). Optimum content of carbon (C) in a *M. aeruginosa* cell is approximately 50% of the cell dry weight (Reynolds, 1984). Thus, the carbon content of our suspension of 500,000 cells/mL of *M. aeruginosa* (suspension with highest cell number) is 11 mg C/L. The highest published chloroform yield for chlorination of *Microcystis* sp. is 61 µg $CHCl_3$/mg C (Huang et al., 2009). Hence, 671 µg/L is the maximum potential THM formation for our *M. aeruginosa* suspension of 500,000 cells/mL. Actual THM yield from the chlorination of IOM and EOM of this cell suspension within a contact time of 24 hours (Table 7.3) is below the maximum THM potential formation values. The published maximum potential yield of HAA precursor for intense *Microcystis* sp. culture is smaller than the published values for chloroform (Huang et al., 2009). However, the actual HAA6 measured in chlorination of IOM and EOM of 500,000 cells/mL of *M. aeruginosa* after 24 hours was below detection limit (Table 7.3).

7.5 Conclusion

Results of this research demonstrate that chlorine doses of 2 to 10 mg/L cause the rapid release and subsequent oxidation of dissolved and cell-bound MCs from *M. aeruginosa*. Additionally, complete toxin release and their subsequent oxidation occurred with maximum 76% reduction in total cell numbers (cell lysis). Detailed investigation of MCs oxidation in ultrapure and natural water in presence of cellular debris revelled surprising data. Kinetic analysis of MCs oxidation in applied chlorination conditions (presence of cellular debris) highlights the differences with previous studies conducted using only dissolved toxin materials. These data provide the novel information on chlorine demand per *M. aeruginosa* cell to obtained cell lysis and toxin release. The cellular chlorine demand beside the toxins chlorination kinetics in presence of cellular debris are the key information provided in this paper to the operators of DWTPs to adjust the chlorination practice for maximum cell lysis and toxin oxidation.

Furthermore, these results demonstrate that chlorination of 500,000 cells/mL and less of *M. aeruginosa* cells do not contribute to the DBP precursor‟s pool. However, water background quality and its DBP formation potentials might influence the direct chlorination of *M. aeruginosa* cells.

7.6 Acknowledgment

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CHAPTER 8 PUBLICATION #4: TOXIC CYANOBACTERIAL BREAKTHROUGH AND ACCUMULATION IN A DRINKING WATER PLANT: A MONITORING AND TREATMENT CHALLENGE

This chapter presents the second manuscript submitted to the journal of WATER RESEARCH 2011. This manuscript presents the novel data on detection of CB and their associated toxins inside a DWTP and in DW.

Toxic cyanobacterial breakthrough and accumulation in a drinking water plant: a monitoring and treatment challenge

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8.1 Abstract

The detection of cyanobacteria and their associated toxin has been intensified in recent year in drinking water sources and the water of drinking water treatment plants (DWTP). The objectives

of this study were to estimate the breakthrough and accumulation of toxic CB in water, scums and sludge inside a DWTP, and to study if chlorination can be an efficient barrier to prevent the breakthrough of cyanotoxins into drinking water. The fate of cyanobacteria and their associated toxins was studied after addition of coagulant and powdered activated carbon, post-clarification, within the clarifier sludge bed, after filtration and final chlorination in a full scale DWTP. Elevated cyanobacterial cell numbers (4.7×106 cells/mL) and total microcystins concentrations up to 10 mg/L were observed in the clarifiers, showing significant accumulation of cyanobacteria in the treatment plant. Cyanobacterial cells from environmental bloom samples were more resistant to chlorination than results obtained with essays using laboratory cultured cells and dissolved standard toxins.

Keyword: Cyanobacteria, Cyanotoxins, Treatment breakthrough, Drinking water

8.2 Introduction

Climate change and population growth contribute to increasing water temperature and nutrient loads and may intensify the proliferation of potentially toxic cyanobacteria (CB) in drinking water (DW) sources (Dale, 2006; Elliott et al., 2006; Jöhnk et al., 2008; Paul, 2008), even in temperate climates (Wiedner et al., 2007). Several CB species are potent producer of a variety of toxins responsible for intermittent but repeated widespread poisoning of wild and domestic animals, fish from aquaculture and humans (Carmichael et al., 2001). The increasing frequency and intensity of cyanobacterial proliferation leading to neurotoxin and hepatotoxin production is a universal problem (Chorus & Bartram, 1999; Merel et al., 2010; Svrcek & Smith, 2004). Reported human health effects of cyanotoxins include gastroenteritis, liver damage, neurotoxic effects and liver cancer. MCs and cylindrospermopsin (CYN) are the most frequently identified toxins but toxins associated with events have not always been identified (Svrcek and Smith 2004; The Cooperative Research Centre for Water Quality and Treatment (Australia), 2007).

Several studies have documented the presence of toxic CB blooms in surface water and at water intakes of drinking water treatment plants (DWTPs) (Carmichael et al., 2001; Lahti, 2001; McQuaid et al. 2011; MDDEP 2008; Merel et al., 2010; Svrcek & Smith, 2004). In a comprehensive national survey including 26 sampling sites over 24 months, maximum

concentrations of ~1,000 µg/L of microcystin-LR (MC-LR) have been reported in French surface water sources (AFSSA-AFSSET, 2006). MCs have been detected in 33 American DW sources but MCs concentrations exceeded 1 µg/l in only 7% of samples (Haddix et al., 2007). Robert et al. (2005) monitored CB and cyanotoxins (Anatoxin, MC-LR, -RR and -YR) every two weeks in the water intakes of six DWTPs located in southeast of Quebec (Canada), including a plant located on Missisquoi Bay (MB) on the Canadian side of the Lake Champlain 24 times for 3 years. MC-LR was found to be the dominant toxin present with maximum concentration of 3.5 µg/L in raw and 0.04 µg/L in treated water (Robert et al., 2005). The MB is prone to cyanobacterial proliferation because of its high nutrient loadings (Simomeau, 2007). Using event-based sampling specifically targeting blooms, McQuaid et al. (2011) reported much higher values of CB and MCs at this water intake with concentration of total intracellular and dissolved MC up to 127 µg/L MC-eq. During the 2004 Wuxi DW crisis in Lake Taihu (China) maximum dissolved MCs concentrations of 35 µg/L were detected in the Meiliang Bay in Lake Taihu (Liu et al. 2011).

The impact of various treatment processes on dissolved cyanotoxins has been thoroughly reviewed and the limitations of various treatment processes are mostly established (Lin et al., 2009; Merel et al., 2010; Svrcek & Smith, 2004; Westrick, 2008; Zamyadi, Ho et al., 2010). However, cyanotoxins are most often cell-bound and represent distinct data available on the fate of CB cells and their associated toxins from real bloom events inside the DWTPs (Henderson et al., 2008; McQuaid et al., 2011). The impact of treatment of cyanobacterial cells is summarized in the Supplementary Data (SD) section Table SD9-*I1*.

The physical removal and potential accumulation of CB cells and aggregates in treatment processes are affected by their size, charge, motility, morphology, and resistance to sheer stress and pressure, and these factors vary widely among species (Dickens & Graham, 1995; Henderson et al., 2008). Bacterial flocs of CB are smaller than 100µm (American Water Works Association (AWWA), 2010). Pumping, coarse screening (> 1mm), grit removal and forced air stripping are not efficient to remove CBs but may lead to cell damage. Micro-screening can be efficient depending on mesh size; a 35 micrometer screen can retain from 40 to 70% of phytoplankton and 10% or less of specific CB, including Microcystis aeruginosa, with no impact on dissolved toxins (Chorus & Bartram, 1999). The impact of cell surface and species-specific coagulant demand on the efficacy of clarification processes has been studied at various scales of

experimentation and recently reviewed (Henderson et al., 2008). Reported efficiencies of CB removal by coagulation/flocculation and sedimentation vary from 62% to 98.9% and are attributed to the low cell density (Henderson et al., 2008). The accumulation of CB and cell bound toxins in water treatment processes is expected and can cause toxin accumulation and subsequent modest release within the treatment plant (Pietsch et al., 2002). Significant accumulation of cell bound MC-LR in gravity filters has been documented at pilot scale using native blooms dominated by Planktothrix rubescens (Pietsch et al., 2002; Schmidt et al., 2009). Rapid sludge removal from sedimentation basins is recommended to ensure that few additional dissolved toxins are released from the sludge beds. Additionally, recycling of sludge drying beds should not be conducted until all toxins have degraded (European Commission, 2005).

Various oxidants including ozone, chlorine, potassium permanganate, chlorine dioxide have been used for the oxidation of CBs resulting in cell damage, oxidation of cell contents and improved removal by filtration (Henderson et al., 2008). The susceptibility of CB cells to oxidation is influenced by the species present, the physiological state of the cells, i.e. the growth phase (Pietsch et al., 2002), and the conditions of oxidation. It has been documented that during oxidation (e.g. chlorination) of a cyanobacterial suspension, the rate of toxin release is determined by the rate of the loss of membrane integrity (Daly et al., 2007; Zamyadi et al., 2010). The efficiency of the oxidative agent in oxidation of released cyanotoxins depends on the toxins present, the concentration of released toxins, the water quality and the contact time (Ho et al., 2010; Rodriguez et al., 2007; Zamyadi et al., 2010).

It must be noted that most studies on the elimination of cyanobacterial cells and toxins have been conducted at laboratory and pilot scale, allowing for the quantification of the impact of water quality and CB speciation. Few research projects have confirmed the anticipated performances at pilot scale and full scale under typical plant operational conditions (Lin et al., 2009; Pietsch et al., 2002; Schmidt et al., 2002). Data available on the fate of CB cells and their associated toxins from real bloom events inside the DWTPs are scarce and preliminary evidence does show their potential breakthrough and accumulation during blooms (Lepisto et al., 1994; Suthaker & Drachenberg, 2007).

Elements of cyanotoxin management plans for drinking water plants include: (a) mandatory guidelines and standards for cyanotoxins in DW, (b) establishment of a plan to monitor CB and

their associated toxins, and (c) treatment methods for removing cyanotoxins (Chorus & Bartram, 1999; Newcombe, 2009). The recommended toxins alert levels are presented in the SD section Table SD9-*I2*. The routine detection of all toxins of relevance to health and water treatment in source water raises several challenges: (1) toxin production is transitory in nature and difficult to predict; (2) the types of toxin(s) produced vary in time and space; (3) the relationship between the number of CB present in water and the probability of the presence of cyanotoxins is complex; and (4) tedious and expensive analytical methods must be used to detect several types of toxins (Codd et al., 2005; Gregor, Marsalek et al., 2007). Based on cyanobacterial cell enumeration and potential cyanotoxin concentration, monitoring strategies for DW (e.g. weekly counts and toxin analysis in DW supplies if 100,000 cells/mL are detected) are applied by different public health organizations (SI section Table SD9-*I3*). *In vivo* measurement of cyanobacterial presence using high frequency monitoring probes can be used to monitor CB in source waters (Ahn et al., 2007; Gregor et al., 2007; McQuaid et al., 2011). McQuaid et al. (2011) proposed a biomass alert level for *in vivo* CB monitoring at the water intake of the DWTP situated in the MB.

The overall objective of this study was to document the accumulation and fate of cyanobacterial cells and their associated toxins in a full scale DWTP coupled with on line *in vivo* monitoring of CBs at the water intake. The purpose was to quantify the vulnerability of DWTPs to peaks of cyanotoxin concentrations resulting from breakthrough and accumulation of toxic CB under realistic operational conditions. This demonstration will help define the scale of monitoring and treatment challenges. The specific objectives of this study were to 1) estimate the accumulation and breakthrough of toxic CB in water, scums and sludge inside a DWTP, and to 2) study if pre- and/or post-chlorination is an efficient barrier to prevent the breakthrough of cyanotoxins into DW.

8.3 Material and Methods

8.3.1 Water source and site description

The water intake of a the water treatment plant located on the MB of Lake Champlain in southern Quebec (Canada) was monitored over three bloom seasons from June to October, 2008, 2009 and 2010. The body of water serves as a source of DW for the surrounding municipalities and as a recreational site for boating, fishing, and swimming. Characteristics of the treatment

processes are presented in Table 9.1. The CB bloom water samples for chlorination experiments were sourced from a bloom event in MB in close proximity of the water intake.

Table 8.1: Characteristics of the treatment process used in the studied DWTP situated at MB (the plant also uses Sodium hydroxide for pH adjustment).

Water Treatment Step	*Water quality& Chemical*	*August 21st, 2008*	*July 14th, 2009*	*July 29th, 2010*	*Specification*
Raw water	Turbidity (NTU)	19.8	15.9	22.9	-
	pH	8.0	7.6	8.6	-
	Temperature (°C)	21.5	20.5	23.7	-
	DO (%)	70.6	75.9	59.4	-
Polymer addition	Hydrex (Silicate) (mg/L)	0.07	0.09	Unknown	-
PAC addition	PAC (mg/L)	9.3	7.1	Not registered	Wood based
Clarifier	-	-	-	-	Efficient clarifier depth: 4.90 m Maximum sludge bed depth: 2.95 m Hydraulic retention time: 55 minutes Solid retention time: 42 hours
Sludge tank for extraction	-	Age: 2 days	Age: less than one day	Unknown	-
Sand-Anthracite Filter	-	-	-	-	Sand effective size: 0.5 mm Sand uniformity coefficient: 1.6 Sand thickness: 15cm Anthracite effective size: 1.0 mm Anthracite uniformity coefficient: 1.7 Anthracite thickness: 61cm
Post-oxidation	Chlorine initial dose (mg/L)	1.2	2.0	Unknown	Minimum contact time 4h
	Chlorine residual in distributed water (mg/L)	0.71	0.84	0.80	-
	Estimative CT (mg.min/L)	117.6	278.4	108.4	-

8.3.2 *In vivo* PC monitoring

An online YSI 6600 V2-4 water-quality multi-probe (YSI, Yellow Springs, Ohio, USA) fitted with an *in vivo* phycocyanin (PC) fluorescence (YSI6131 Blue Green Algae) self cleaning „„wiped"" probe was used in this study (YSI Incorporated, 2006). The PC probe was installed inside the DWTP at the raw water intake prior to any treatment and measurements recorded every 30 minutes. The PC probe excites the CB PC at 590 nm (with a pass-band of 565–605 nm) and measures the pigment"s emission at 660 nm. The probe detects light emitted in the range of 640–680 nm. The probe raw readings are presented in ratio fluorescent units (RFU). The probe resolution, detection limit and limit of quantification is 0.1 RFU over its range of 0–100 RFU, 0.2 RFU) and 0.7 RFU, respectively (McQuaid et al., 2011).

8.3.3 Sampling procedure

Weekly field visits were conducted on a fixed day to collect probe records and water samples were taken from the water intake only for microscopic taxonomy and toxin analysis. If RFU augmentations were observed in the probe readings within 24 hours prior to the sampling day or the presence of CB was detected inside the DWTP, triplicate water samples were taken across the treatment processes from raw to treated water: raw water from the pipeline conducting raw water to the treatment facility prior to any treatment; surface of the reactive addition and flash mix tank; scum at the surface of the clarifier/sedimentation tank; sludge from the sludge bed of the clarifier using the sampling valve; concentrated sludge in the concentration cone using the sampling valve; scum over the filter; and treated water at the outlet of the reservoir (complete Cl_2 contact time). Sampling taps were fully flushed before taking samples. Samples for taxonomic counts were preserved using Lugol‟s iodine and samples for total toxins analysis were stored in -25^{o}C.

8.3.4 Field samples pre-treatment

Cells from all field samples were submitted to three freeze/thaw cycles before any analysis for total toxins. In 2010, field samples (5mL) were vortexed in conical centrifuge tubes (BD Falcon, 50mL, Fisher Scientific) at 3000 rpm for 2 minutes, treated with internal standard (25µL of 100µg/L nodularin (in methanol, providing final nodularin concentration of 500ng/L) and passed through syringe filter (10mL polypropylene syringes and 25mm diameter Whatman GD/X GMF, 0.45µm pore size filters) then divided into 3 separate 2mL amber autosampler vials. For this set of samples (an initial first test of the method with real samples) extracts were first analyzed as described. If concentrations exceeded the highest concentration in the calibration standard curve, subsequent dilutions were completed with appropriate addition of internal standard before analysis.

8.3.5 Chlorination experiments

Liquid chlorine stock solution was purchased from Fischer (USA). Chlorine stock and chlorine residual concentrations were determined using the DPD-FAS titration method described in Standard Methods (American Public Health Association (APHA), 1998). Typical chlorine stock solutions concentration is 50,000 mg/L as free chlorine. The experimental plan included

chlorination of a sample of CB bloom from MB. A chlorine decay experiment was conducted prior to each experiment. For chlorine decay experiments, water samples were dosed with the desired chlorine concentration and triplicate samples taken at specified contact times for free chlorine residual determinations using the DPD-FAS titration method. CB cells exposure to chlorine is in CT, the residual concentration of the chlorine multiplied by time (Ho et al., 2006). All chlorination experiments were conducted in triplicate in brown amber bottles at room temperature (20 ± 2oC).

Environmental CB bloom samples were taken from MB (5.7×10^5 (±4%) cells/mL) where the speciation of the CB population was conducted. Chlorine was added from the chlorine stock solution (2 and 5 mg/L). Samples were quenched at specified contact times with sodium thiosulphate at a stoichiometric ratio specified in Standard Methods (APHA, 1998). To determine cell lysis total cell microscopic counts were conducted in triplicate on 2-mL samples after preservation in Lugol‟s iodine (Lund et al., 1958; Wetzel, 2000). Remaining quenched water samples were divided in two subsamples of 250 mL: the first subsample was immediately filtered through 0.45 μm cellulose filter (Whatman, UK) to remove cellular material, and then analyzed for extracellular toxins. The second subsample was subjected to three freeze/thaw cycles before filtration over 0.45 μm filter to analyze for total toxins (both the cell-bound and extracellular). For all experiments the extracellular concentrations of toxins analysis were always below their total concentration. The difference between total and extracellular levels of toxins was estimated as the cell-bound concentration.

8.3.6 Chemicals and reagents

Standard solutions of MC-LR, cylindrospermopsin, MC-RR, nodularin, dm MC-LR were purchased from National Research Council (Halifax, NS). Anatoxin-a was purchased as anatoxin-a fumarate from Cedarlane (Burlington, ON) and MC-LF, MC-LW, MC-LY, MC-YR were all purchased from DHI (Hørsholm, Denmark). Individual toxins were diluted to 1 mg/L with HPLC-grade MeOH and kept in amber vials at -20°C. Working solutions with all toxins for external calibration (10, 50, 100, 250, 500, 1000, 5000, 10000ng/L each with 500ng/L internal standard) were prepared via appropriate dilutions with HPLC grade H_2O and were kept at 4°C prior to and during analysis. All chemicals and reagents were analytical grade from various suppliers.

8.3.7 Analysis of toxins

For the 2008, 2009 and chlorination experiment samples MC-LR analyses were conducted using an Abraxis Microcystin ELISA Plate (an enzyme-linked immunosorbent assay – Abraxis LLC, Pennsylvania, USA). The analyses of MC-LR concentrations were measured as µg/L of MC-LR eq. The accuracy of the Plate was validated using standard toxin materials (data not shown).

The 2010 toxin analysis was carried out via online solid phase extraction-liquid chromatography coupled to tandem mass spectrometry (online SPE-LC-MS/MS) consisting of a CTC Autosampler (from Thermo by Leap Technologies), a Thermo Fisher (Waltham, MA, USA) HPLC system (with Accela 1250/analytical and 600/loading pumps), one 6- and one 10-port switching valve (VICI), a HotPocket column heater (Thermo Fisher) and a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher) with an Ion Max atmospheric pressure ionization (API) source (Thermo Fisher). The method, adapted from published multi-toxin (Dell'Aversano et al., 2004; Oehrle et al., 2010; Spoof et al., 2010) and online SPE-LC-MS/MS methods (Segura et al., 2007; Stoob et al., 2005), allowed for determination of ng/L toxin concentrations in 1 mL samples without arduous pre-concentration. The runtime was 6.8 minutes/sample with recoveries ranging from 97-109%. Hypersil GOLD (Thermo Fisher, Waltham MA USA) LC columns were employed in online extraction (12µm, 20 × 2.1mm) and analyte separation (1.9µm, 50 x 2.1mm) with a stepped gradient of formic acid and acetonitrile as the mobile phase.

8.4 Results and discussion

8.4.1 Breakthrough and accumulation of toxic CB inside a DWTP

On the afternoon of the August 20th, 2008 a notable increase of CB was recorded by the *in vivo* PC probe (Figure 8.1a). Raw water at the water intake of the DWTP contained 7.70×10^5 cells/mL of CB and 6.1 µg/L of MC-LR eq. *Microcystis* sp., *Anabaena* sp., *Pseudanabaena* sp., *Aphanothece* sp., *Aphanocapsa* sp., *Glaucospira* sp., *Planktolyngbya* sp. and *Aphanizomenon* sp. were identified in this sample with *Microcystis aeruginosa* being the dominant species representing 61% and *Anabaena* representing 33% of the total CB biovolume. The flux of CB cells into the DWTP decreased on August 21st, 2008 as shown by probe readings and a CB count of 2.01×10^5 cells/mL with *M. aeruginosa* species representing 74.5% and *Anabaena* sp. approximately 16% of the total CB biovolume (Figure 8.1b). McQuaid et al. (2011) proposed an

alert level of 2.4 RFU (equivalent of 1 mm^3/L total CB biovolume and maximum potential of 13 µg/L MC-LR) for this source water *in vivo* PC probe. For these two days, the *in vivo* fluorescence measurements in raw water exceeded the proposed alert level while total cell-bound actual dissolved toxins remained much lower than the calculated maximum production potential.

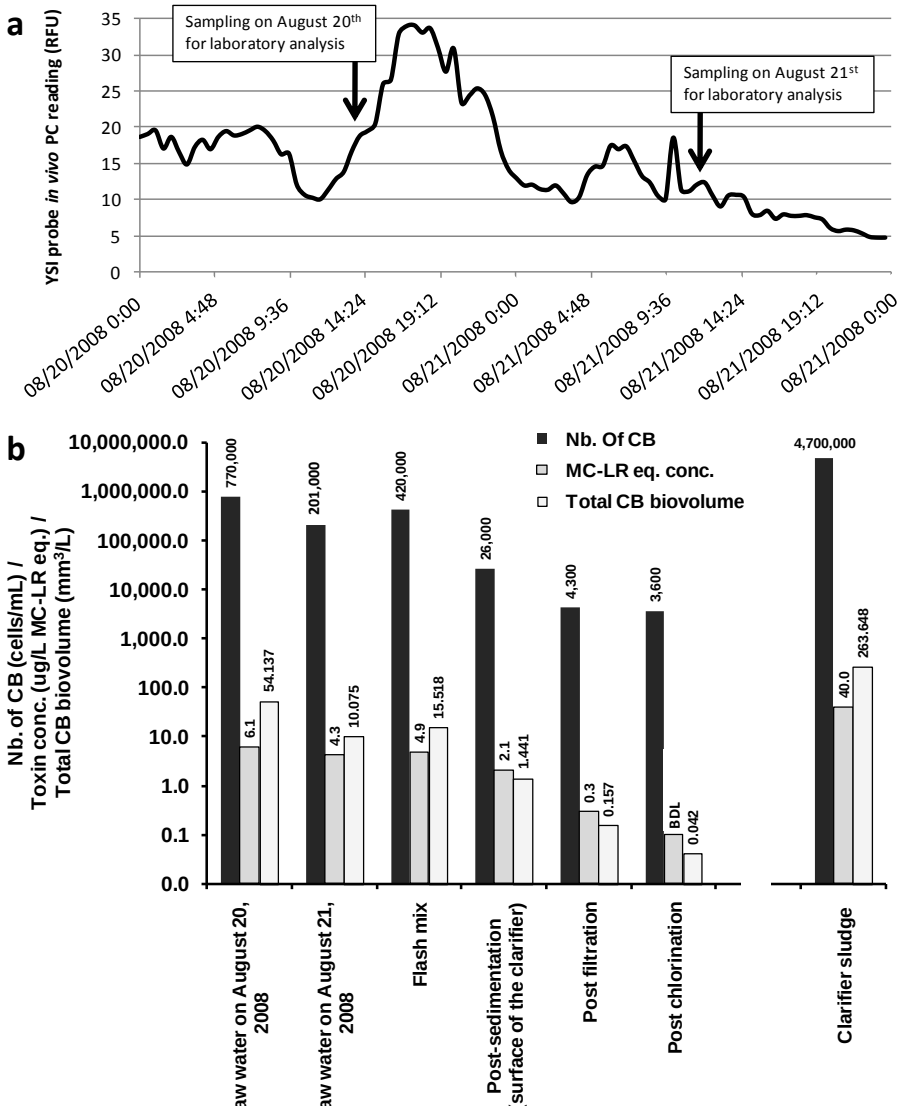

Figure 8.1: Breakthrough and accumulation of CB cells in the studied DWTP: (a) *in vivo* intensive monitoring at the raw water (arrow points sampling time for laboratory analysis) with the x axes in month/day/year hour: minute, and (b) cell counts, toxin analysis and total CB biovolume estimation in raw water (August 20th and 21st, 2008) and after different treatment process (August 21st, 2008). (BDL: Below Detection Limit)

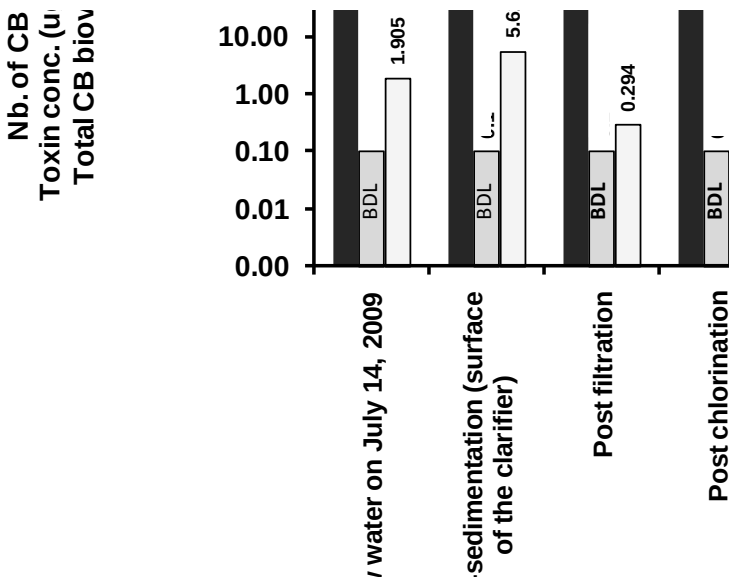

Figure 8.2: Breakthrough and accumulation of CB cells in the studied DWTP on July 2009: (a) in vivo intensive monitoring at the raw water (arrow points sampling time for laboratory analysis) with the x axes in month/day/year hour:minute, and (b) cell counts, toxin analysis and total CB biovolume estimation after different treatment processes on July 14th, 2009.

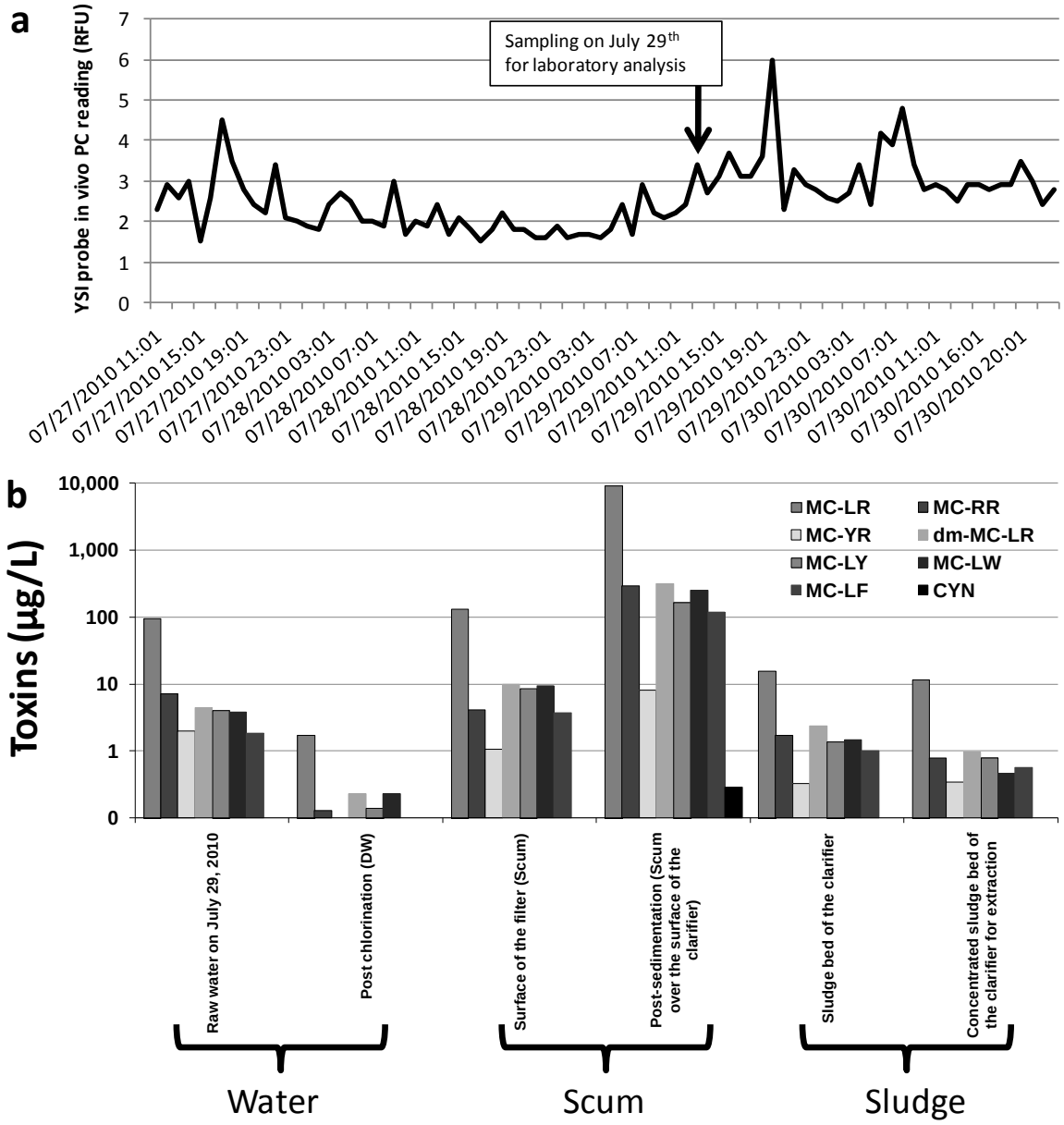

Figure 8.3: Breakthrough and accumulation of CB cells on July 2010: (a) *in vivo* intensive monitoring at the raw water (arrow points sampling time for laboratory analysis) with the x axes in month/day/year hour:minute, and (b) cell counts and toxin analysis after different treatment processes on July 29th, 2010.

Table 8.2: Cumulative percentage of trapped cell after each treatment process with their estimated total biovolume from microscopic taxonomy

Treatment process	August 21st, 2008				July 14th, 2009			
	APHAZ+APHAC*		MIC+ANA**		APHAZ+APHAC		MIC+ANA	
	Biovolume (mm^3/L)	% of accumulative removal	Biovolume (mm^3/L)	% of accumulative removal	Biovolume (mm^3/L)	% of accumulative removal	Biovolume (mm^3/L)	% of accumulative removal
Post-clarifier	1.103	-56.0	0.321	96.5	3.228	-931	2.401	-51.4
Post-filtration	0.101	85.0	0.053	99.4	0.229	26.8	0.064	96
Post-chlorination	0.020	97.9	0.015	99.7	0.001	99.3	0.000	100

* *Aphanizomenon* sp. (APHAZ) + *Aphanothece* sp. (APHAC)

** *Microcystis* sp. (MIC) + *Anabaena* sp. (ANA)

During both days, the cumulative flux of coagulated CB cells to the clarifier resulted in multi-fold accumulation (4.70×10^6 cell/mL) of toxic CB corresponding to a significant mass of 4.92g MC-LR eq. present in the total volume of the sludge bed of the clarifier (Figure 8.1b). As the solids retention time is estimated at about 42 hours (2% purge) the accumulation of cells in the sludge should reflect the composition and toxin content of the incoming CBs, unless selective removal of certain species occurs. Interestingly, the cell speciation in the sludge bed did remain similar with a dominance of *Microcystis* sp. (82% of biovolume) and *Anabaena* sp. (14% of biovolume) and a relatively constant toxin cell content of 0.08 pg MCs eq./cell. The sludge blanket clarifier removed 86% of the total CB cell number present in the raw water and reduced total toxins to 2.1 µg/L of MC-LR eq. Although the same CB species were identified in post-clarifier water, a shift in the most abundant species was observed with a dominance of *Aphanizomenon* sp. (1.2×10^4 cells/mL, 76.3% of biovolume) and *Aphanothece* sp. (4.7×10^3 cells/mL, 2.4% of biovolume). Table 8.2, shows that clarifier efficiency in removal of CB total biovolume is species dependent. Dual media filtration further reduced the CB cells to 4.3×10^3cells/mL (0.157 mm^3/L) with *Anabaena flos-aquae* and *Aphanothece minutissima* remaining the most abundant species. However, in filtered water, small concentrations of 7.3×10^2 of *Microcystis* sp. still represented 32% of the total biovolume because of its elevated cell size. Post chlorination with a minimum CT of 117 mg.min/L was not efficient in lysing the CB cells with a modest 16% reduction in the remaining cell numbers and elimination of MC below the detection limit (0.1µg/L). However the average cell biovolume was considerably

reduced from 57 to 12 μm^3/cell as a result of the preferential lysis of the remaining *Anabaena* sp. and *Microcystis* sp.

Figure 8.2a presents the increasing flux of CB cells into the DWTP from early morning hours of the July 12th, 2009. The *in vivo* PC probe readings exceeded the proposed alert level of 2.4 RFU on July 13th, 2009 (Figure 8.2a). On July 14th 2009, the dominant CB in the raw water was *Microcystis aeruginosa* (2.5×10^4 cells/mL, 1.6 mm^3/L) but also included *Aphanothece clathrata brevis* (2.4×10^4 cells/mL, 0.013 mm^3/L), *Aphanocapsa delicatissima* (1.8×10^4 cells/mL, 0.007 mm^3/L), and *Aphanizomenon flos-aquae* (4.0×10^3 cells/mL, 0.300 mm^3/L) (Figure 8.2b). The toxin production of these CB cells was below the detection limit.

As also occurred in 2008 (Figure 8.1b) the cumulative flux of coagulated and flocculated CB cells to the clarifier caused major CB cells accumulation in the sludge bed of the clarifier (2.4×10^5 cells/mL – Figure 8.2b). *Microcystis aeruginosa* was the dominant species (84.51% of the total biovolume) in the settled CB cell (in sludge) followed by *Aphanizomenon flos-aquae* (15.48% of the total biovolume) and *Aphanothece clathrata brevis* (0.01% of the total biovolume). The MCs concentration in the sludge bed was 1.5 MC-LR eq. and *Microcystis aeruginosa* was the dominant species over the clarifier followed by *Aphanizomenon flos-aquae* and *Aphanothece clathrata brevis*. However the total biovolume of small-sized *Aphanothece clathrata brevis* (0.5 μm^3/cell) over the clarifier was approximately two times greater than these cells total biovolume in the settled sludge. This outcome is in complete coherence with the -931% removal of the total biovolume of this species after clarifier (Table 8.2). *Aphanothece clathrata brevis* was the dominant species in post-filtration water when considering total cell numbers. However, 76.7% of the total biovolume in filtered water consisted of *Aphanizomenon flos-aquae*, followed by *Microcystis aeruginosa* (21.9%) and *Aphanothece clathrata brevis* (1.4%). These results highlight the importance of presenting the results in a scale unit that has a better coherence with the reality (i.e. biovolume rather than cell numbers). *Aphanothece clathrata brevis* was the only species detected in the chlorinated water (Figure 8.2b).

These results are in agreement with the CB removal efficiencies (62-98.9%) of coagulation/flocculation and sedimentation processes published previously (Henderson et al., 2008). Coagulation can be optimized with an online system measuring the zeta potential (Bernhardt & Schell, 1993; Bernhardt & Clasen, 1994; Lai et al., 2002). Our observations

provide novel data on CB cell breakthrough in dual media sand-anthracite filter used in this DWTP (Figure 8.1b and 8.2b). CB cells were also detected in chlorinated water for both years as previously reported for *Anabaena circinalis* (Zamyadi et al., 2010). However, cell integrity analysis showed that all of those cells were metabolically inactivated (Zamyadi et al., 2010).

In 2008 and 2009 the breakthrough and accumulation of CB cells in the DWTP was caused by an influx of high CB cell numbers into the facility as shown by parallel high *in vivo* peaks (Figure 8.1 and 8.2). However, toxin production did not in any way follow the same patterns. In 2010, even though *in vivo* CB cell measurements were lower than the previous years (Figure 8.3a) the unit cell toxin production was higher when compared to previous years (Figure 8.3b). The proportion of different cyanotoxins present in water samples are also presented in Figure 8.3b. MC-LR remained the dominant toxin but observing traces of CYN is of great interest (Figure 8.3b).

The total MCs concentration at the water intake of the DWTP reached 118.70 µg/L. PAC has been shown to be efficient to remove dissolved MCs and CYN (Ho et al., 2011). Significant removal of MCs has been observed during the pre-treatment stage in DWTPs using PAC addition to the raw water (AWWA, 2010). However, the PAC dose, the PAC type, the water quality and the type of cyanotoxin influence its efficacy. PAC efficiencies can vary from 20 to 80% toxin removal for a dose of approximately 10 mg/L of PAC (Ho et al., 2011). In the studied DWTP even though PAC was added to the raw water, high toxin concentrations were detected after the addition of PAC. PAC does not remove intact cells and cell-bound toxins.

A green scum was observed over the clarifier and 10,300 µg/L of MCs and 0.29 of µg/L of CYN were detected in the scum sample (Figure 8.3b). The presence of a green scum at the surface of the clarifier confirms the breakthrough from the sludge bed and their co-current movement, accumulation and potential development over the settled water collecting pipes. Table 8.2 shows that this phenomenon had also occurred in 2008 and 2009. The negative removal of total biovolume of CB cells in clarified water (Table 8.2) is due to release of CB cells from sludge. Poly-aluminum chloride is used as coagulant in this DWTP. Recent studies have observed coagulation inhibition caused by surface-related organic matter and proteins derived from *Microcystis aeruginosa* while using Poly-aluminum chloride (Sano et al., 2011; Takaara et al., 2007; Takaara et al., 2010). Furthermore, during the cyanobacterial proliferation period, the flocs

are light. It is therefore recommended that coagulant aids, e.g. anion polymers, be used for the flocculation process (Bernhardt & Clasen, 1994). In certain cases, the use of a flocculating agent containing cationic (AFSSA-AFSSET, 2006) or anionic (Bernhardt & Clasen, 1993) polymers would allow a better elimination of the CB. Furthermore, aluminum sulfate can be a more efficient flocculating agent than iron salts (Pietsch et al., 2002). Significant cyanotoxin release during flocculation/filtration has been reported (Pietsch et al., 2002). Hydraulic stress (turbulence) caused by flocculation/filtration and pressure gradients in pipes and filters have a destabilizing effect on the integrity of the cyanobacterial cells. However, the stability of cyanobacterial cells varies according to their growth phase, thus, depending on their growth phase, flocculation/filtration may lead to the release of toxins (Pietsch et al., 2002). Pietsch et al. (2002) showed that cells captured in the clarifiers can release all cyanotoxins within the first 48 hours in clarifiers with solid retention time of over 48 hours. The estimated sludge retention time in the clarifiers in this study was approximately 42 hours (Table 8.1). CB lysis retained on the filters can occur after 24 to 48 hours of retention and good management of the filters maintenance process is paramount (Chorus & Bartram, 1999; Lepisto et al., 1994).

Total MCs concentration in chlorinated DW reached 2.47 µg/L (Figure 8.3b) exceeding the recommended MCs alert levels for DW (SD section Table SD8-*I2*). MC-LR was the dominant analog with the highest concentration (1.74 µg/L). Results from laboratory scale chlorination experiments are contradictory. While chlorination of dissolved MCs in ultrapure and natural water was efficient (Ho et al., 2010; Rodriguez et al., 2007), Daly et al. (2007) observed 14.1% to 94.1% removal of total MCs produced by *Microcystis aeruginosa* cells. Thus, 2010 toxins monitoring demonstrates that it is essential to verify the chlorination efficiency in the presence of high concentrations of toxins and cellular materials, and questions the strength of chlorination as a barrier as predicted by laboratory kinetic oxidation trials.

A total MCs concentration of 15.5 µg/L was measured in the concentrated sludge of the clarifier (in the sludge concentrator for extraction). Toxic CB management in the DWTP must also consider the permanent elimination and/or reuse of the sludge. Sludge treatment needs particular attention as intracellular toxins might be released during sludge handling and supernatant recycling could increase the loads of toxins for treatment.

The recent published guidelines recommend CB biweekly monitoring at alert level 3 (65,000 cells/mL) (Newcombe, 2009; Newcombe et al., 2010). Our observations suggest that low frequency sampling may lead to the underestimation of significant peak toxic CB influxes into the DWTP. Our results also suggest that lower CB influx accumulating over a long period in the clarifier could also lead to significant accumulation and potential release. Figure 8.1b and 8.2b show that the breakthrough of CB cells through post-chlorination is possible under these conditions.

8.4.2 Chlorination of MB CB bloom water sample

8.4.2.1 Impact of CB cells and cell bound toxins on chlorine decay.

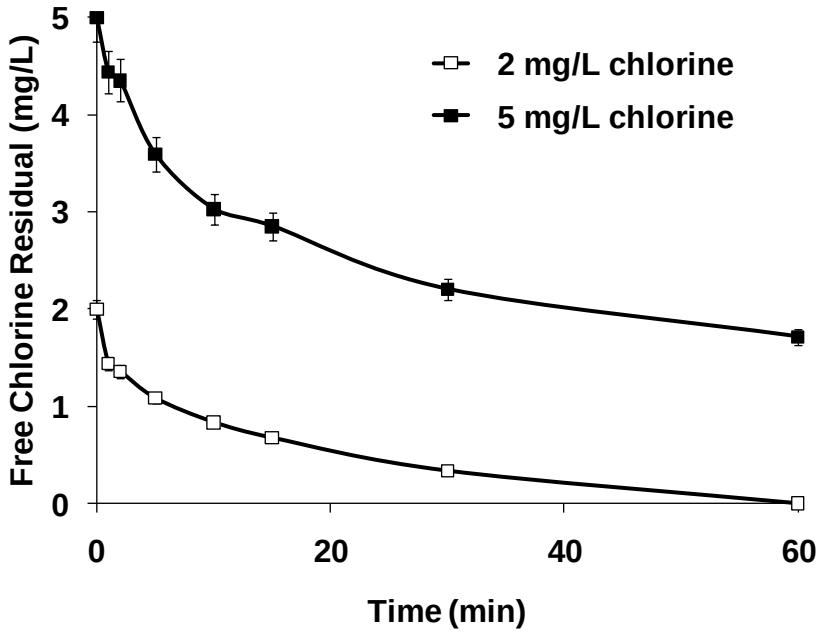

Figure 8.4: Chlorine decay in Missisquoi Bay (MB) bloom water samples with 5.7×10^5 (±4%) cells/mL at ambient pH (pH before chlorination was 7.8; pH augmented to 8.1 and 8.3 after chlorination with 2 and 5 mg/L Cl_2 respectively, and remained constant for the rest of the experiment).

Free chlorine decay and microscopic cell counts were conducted using the MB CB bloom samples at cell number of approximately 5.7×10^5 (±4%) cells/mL, at two chlorine doses, 2 and 5

mg/L as Cl_2 (Figure 8.4). The standard deviation value of these cell counts (triplicate count per sample) fit within the confidence limit of cell enumeration methods (Laslett et al., 1997; Lund et al., 1958) (data not shown). CT values were calculated from chlorine decay data by fitting a two pathway integration model to a two phase decay curve: fast (<2 minutes) and slow (Sohn et al., 2004). The resulting first order rate constants for 2 and 5 mg/L of chlorine decay are 1.1×10^{-3} (r^2 = 0.92) and 1.0×10^{-3} (r^2 = 0.97), respectively.

8.4.2.2 Effect of chlorination on CB cells and toxin release

The response of a CB bloom sample from MB of approximately 5.7×10^5 (±4%) cells/mL to chlorine doses of 2 and 5 mg/L at ambient pH (8.3) was evaluated. For these bloom samples, 28% to 53% reductions of total number of cells, corresponding to full cell lysis, were observed at the end of the chlorination period for both chlorine doses (Figure8.5). However it is not possible to categorize the remaining cells as damaged membrane or metabolically inactive cells as cell-bound toxin are still observed even after the highest CT (148.26 mg.min/L) (Figure 8.6). In previous studies (Daly et al., 2007; Lin et al., 2009; Zamyadi et al., 2010), fast release of cell-bound compounds have been observed when pre-chlorinating (with low chlorine doses, 1-5 mg/L Cl_2) natural water loaded with *Anabaena* sp. and *Microcystis* sp. However this study presents the first set of experiments using natural bloom samples incorporating seven different CB species present but dominated by *Microcystis* sp (Figure 8.7). Furthermore, after the chlorination with a CT of 148 mg.min/L and a 53% reduction in total cell number the proportion of CB species with regards to total number of cells present was almost the same as before chlorination with slight declines in *Anabaena* sp. and *Aphanothece* sp (Figure 8.7).

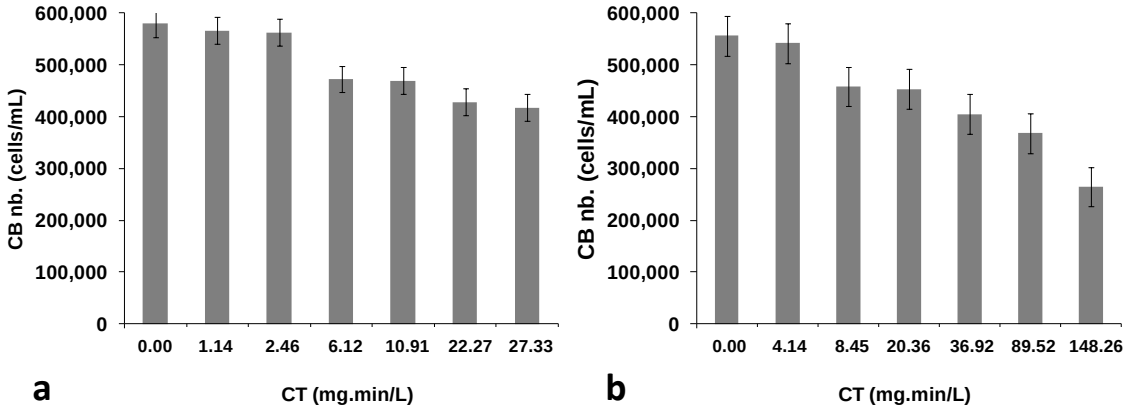

Figure 8.5: Cell numbers after chlorination of MB bloom water samples using (a) 2 mg/L Cl_2, and (b) 5 mg/L Cl_2.

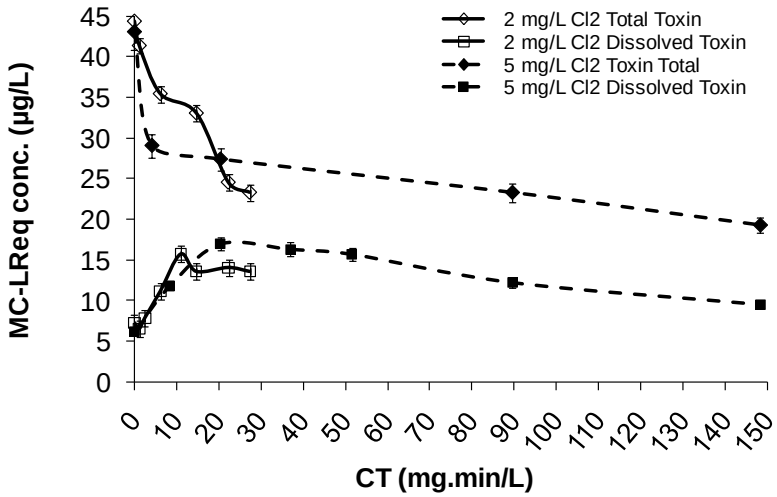

Figure 8.6: Toxins release and oxidation after chlorination of MB bloom water samples using 2 and 5 mg/L Cl_2.

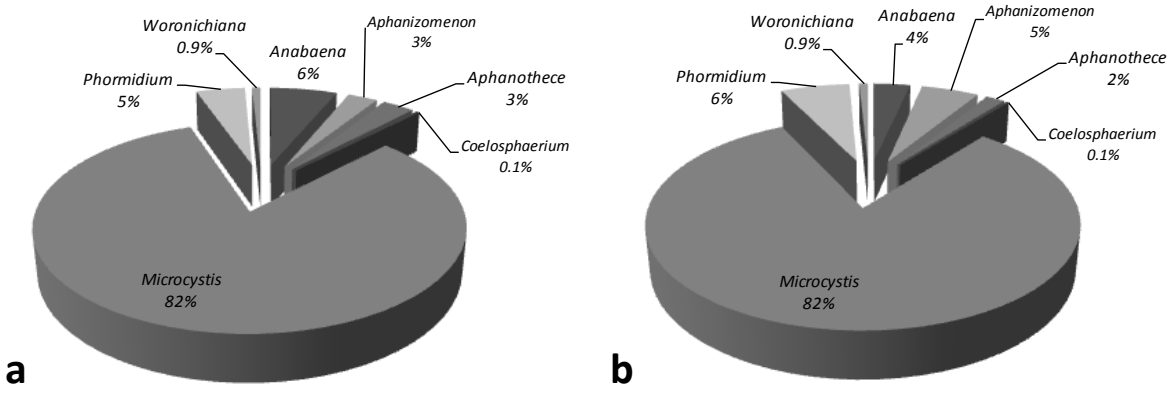

Figure 8.7: Proportion of seven CB species out of total CB cell in MB bloom sample (a) before chlorination (5.7×10^5 (±4%) cells/mL), and (b) after chlorination using a CT of 148 mg.min/L (2.7×10^5 (±4%) cells/mL – 53% reduction of total cell numbers).

It is possible to assume a first-order reaction with regards to chlorine reaction and the reduction of total cell numbers (Daly et al., 2007; Lin et al., 2009). Thus the reaction between chlorine and the cells is assumed to be of second order and the rate at which cell lysis occurs (k_{lysis}) is evaluated using equation 1: .

$$Ln\left(\frac{[N_{CT}]}{[N_0]}\right) = -k_{lysis} \times CT \qquad \text{eq. 1}$$

where CT equals the chlorine exposure; N_{CT} equals the number of lysed cells after a given chlorine exposure; N_0 equals the number of cells at CT=0; and k_{lysis} equals the rate at which cell oxidation occurs (Zamyadi et al., 2010). The evaluated rates for cell reaction with chlorine from this study are in disagreement with the results of previous studies (Table 8.3). The vulnerability of the cells of the CB species to chlorination is influenced by the physiological state of the cells, cell densities, water matrix and the chlorination conditions (Hart et al., 1998; Lin et al., 2009; Pietsch et al., 2002).

Table 8.3: CB k_{lysis} ($M^{-1}s^{-1}$) derived from this study compared to published results (Daly et al., 2007; Ding et al., 2010; Lin et al., 2009; Zamyadi et al., 2010). Comments in parenthesis describe the origin of the CB cells (from laboratory cultures or environmental bloom samples).

This Study	2 mg/L Cl_2	5 mg/L Cl_2	Published papers	Daly et al. 2007	Lin et al. 2009	Zamyadi et al. 2010	Ding et al. 2010
CB species	Ambient pH (8.3)		CB species	pH 6.8-7.6	pH 8.3-8.6	pH 6.8-8	pH 7.6
MB bloom sample with 7 species present	15.97	3.78	*A. circinalis*		1400-3400 (culture)	1400-1600	-
			M. aeruginosa	670±77	790-1100 (culture), 70-590 (bloom)	-	55.9

8.5 Conclusion

Sampling once or twice a week on fixed day fails to spot the highly temporal variability of CB bloom presence at the water intake of the DWTP. Non-event based sampling causes an underestimation of the transitory CB bloom events and the accumulation and breakthrough of toxic CB in the DWTP. A monitoring strategy including intensive *in vivo* fluorescence measurements and event-based sampling would help to prevent the documented event of 2008-2010. DWTP operators should consider the details of the operational facilities before interpreting laboratory derived data for real environmental conditions. This paper provides the novel information on accumulation of potentially toxic CB cells inside DWTPs, particularly the clarification and filtration processes. Future systematic studies of these phenomena in full scale operational DWTPs are required to establish the key factors for CB accumulation and removal, and prepare efficient management plans.

Furthermore, our findings demonstrate that breakthrough of cyanotoxins at concentration exceeding the health-based exposure alert levels can occur, even for toxins considered to be readily oxidized by chlorine. Finally, the extrapolation of laboratory scale chlorination experiments based on dissolved toxins and cultured CB species to the operational conditions requires further investigation.

8.6 Acknowledgment

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8.8 Supplementary data (SD)

Toxic cyanobacterial breakthrough and accumulation in a drinking water plant: a monitoring and treatment challenge

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Supplementary data (SD)

Number of pages: 6

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Number of table: 3

Table SD8.*I1*: Treatment processes for the removal of cyanobacterial cells and cell-bound cyanotoxins

(Carrière et al., 2010; Chorus & Bartram, 1999; Cook & Newcombe, 2002; Hall et al., 2000; Lin et al., 2009; Lionel et al., 2011; Maatouk et al., 2002; Merel et al., 2010; Montiel & Welté, 1998; Newcombe & Nicholson, 2004; Pietsch et al., 2002; Sano et al., 2011; Svrcek & Smith, 2004; Takaara et al., 2010; The Cooperative Research Centre for Water Quality and Treatment (Australia), 2007; Warren et al., 2010; Zamyadi et al., 2010).

Treatment process	Scale	Treatment efficiency
Coagulation/flocculation clarification	Pilot, Laboratory (Lab) & Full	Effective for the removal of intracellular toxins. Different cell removal efficiencies observed for different coagulants. Organic matter of certain *M. aeruginosa* can inhibit coagulation depending on the coagulant. Coagulation inhabitation observed the growth phase of phase of the cell impacts the removal efficiency. Accumulation of the removed cells in sludge bed seen as a way of isolating toxic cell from the water. Long retention time (clarifiers with permanent sludge blanket) favours the CB cells removal. High cell numbers might increase coagulant demand.
Rapid filtration	Pilot & Lab	Effective for the removal of intracellular toxins. Major cells accumulation but cells are not allowed to accumulate on filter for prolonged periods. High cell numbers might cause excessive head loss development, filter clogging, short filter runs and causing an increase of up to 20% of water losses in backwashes.
Slow sand filtration	Pilot & Lab	As for rapid sand filtration, with additional possibility of biological degradation of dissolved toxins
Membrane processes	Lab	Very effective for the removal of intracellular toxins. Provided cells are not allowed to accumulate on membrane for prolonged periods
Dissolved air flotation (DAF)	Pilot & Lab	Flotation gives better results than decantation for the separation of low-density floc particles, e.g. CB cells. DAF is found to be more effective than decantation in the removal of CB cells including *Microcystis*. Toxins release during DAF process and sludge treatment is an issue to be considered
Oxidation processes	Pilot & Lab	The efficiency of pre-oxidation depends on cell number, present species, toxins concentration, oxidant dose and contact time. Pre-oxidation induce cell membrane damage and/or lysis and consequent increase in dissolved toxin/taste & odor compounds levels and disinfection by products formation. If applied under controlled situation it might be efficient; pre-oxidation of cells will prevent process disturbances in DWTP due to presence of CB cells.
Ozonation	Pilot & Lab	Ozonation is effective for all cells but not all cell-bound toxins including the saxitoxins.
Chlorination	Pilot & Lab	Recent data provide reliable evidence that chlorination is effective for cell-bound saxitoxins oxidation at pH 6.8 and 8. In direct chlorination of potentially toxic CB cells, the disinfection by products formation is mainly influenced by the water quality background
Chloramination	Pilot & Lab	Ineffective for released toxins removal
Chlorine dioxide	Lab	Not effective for oxidation of released toxins with doses used in DW treatment
Potassium permanganate	Pilot & Lab	Effective for released MC, anatoxins and CYN, no data for saxitoxins
Hydrogen peroxide	Lab	Not effective on its own
UV Radiation	Lab	Induce cell membrane damage, toxin release, and toxins oxidation occurs at impractically high doses or in the presence of a catalyst
Adsorption – Powder Activated Carbon (PAC)	Pilot & Lab	Has no documented impact on cell viability or their removal. Efficient for removal of released: MC, CYN and saxitoxins The type of PAC, dose of PAC, water quality including pH and present toxins plays an important role in removal by PAC.
Adsorption – Granular Activated Carbon (GAC)	Lab	Physical removal of CB cells during filtration and simultaneous removal of cell-bound toxins within intact cells. High doses required for toxins. GAC adsorption of release toxins displays a limited lifetime varying between 2 months to more than one year depending on the type of toxin and the water quality.
Biological filtration	Pilot & Lab	When functioning at the optimum this process can be very effective for the removal of intact cells and most of released toxins. However, factors affecting the removal such as biofilm mass and composition, acclimation periods, temperature and water quality cannot be easily controlled
Membrane Processes	Lab	Very efficient for removal of CB cells and cell-bound toxins within intact cells. However, accumulation of cell over the filter and hydraulic stress during the back wash might cause toxins release. Released toxins removal varies depending on the membrane pore size distribution.

Table SD8.*I2*: The main cyanotoxins of interest, their toxicity, CB species producing these toxins and water quality guidelines and standards (Catterall, 1980; Ellis, 2009; Höger, 2003; Humpage, 2008; Merel et al., 2010; Newcombe et al., 2010; Svrcek & Smith, 2004).

Cyanotoxin	LD_{50}* (i.p. mouse µg/kg body weight)	CB species known to produce the toxins	Mechanism of toxicity	Chemical structure	Health-based exposure guidelines/recommended alert levels
Microcystins (MCs) in general (~60 known analogues) MC-LR MC-LA MC-YR MC-RR	45 - >1000 50 (25-125) 50 70 300 - 600	*Microcystis, Anabaena, Planktothrix, Oscillatoria, Nostoc, Hapalosiphon, Anabaenopsis, Aphanizomenon*	Hepatotoxic: Protein phosphatase blockers by covalent binding and cause haemorrhaging of the liver	Cyclic peptides with the amino acid ADDA	South Africa: 0.8 µg/L MC-LR World Health Organization (WHO), Czech Republic, China, France, Italy, Japan, Korea, New Zealand, Norway, Poland: 1 µg/L MC-LR Brazil, Spain: 1 µg/L MCs Australia: 1.3 µg/L MC-LR toxicity equivalent Canada (Enviroment Canada): 1.5 µg/L MC-LR toxicity Quebec (Canada): 1.5 µg/L MC-LR toxicity
Saxitoxins (STXs) also known as paralytic shellfish poisons in general (~30 known analogues) Saxitoxin (STX) C-toxin1 & 2(C1 & C2) Gonyautoxin2 & 3 (GTX2 & GTX3)	10 - 30	*Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya*	Neurotoxin: STXs are potent voltage-gated sodium channel antagonists, causing numbness, paralysis and death by respiratory arrest. STX disrupts the nervous system via binding to the sodium channel and inhibits the sodium ions transport	Carbamate alkaloids ($C_{10}H_{17}N_7O_4$) based on a 3,4,6-trialkyl tetrahydropurine skeleton which can be further carbamylated, sulphated or N-sulphocarbamylated: non-sulphated STX, singly sulphated GTX, doubly sulphated C	Australia, Brazil & New Zealand: 3.0 µg/L STX toxicity equivalent
Cylindrospermopsin (CYL)	200 – 2100	*Cylindrospermopsis, Aphanizomenon, Umezakia, Raphidiopsis, Anabaena*	Cytotoxic: blocks protein synthesis	Cyclic guanidine alkaloid	New Zealand: 1 µg/L Brazil: 15 µg/L
Anatoxin Anatoxin-a Anatoxin-a(S)	 200 – 250 20 – 40	*Anabaena* sp., *Aphanizomenon* sp., *Oscillatoria* sp., *Cylindrospermopsis* sp., *Lyngbya* sp., *Schizothrix* sp.	Neurotoxic: Acute poisoning results in death by paralysis and respiratory failure. Acute toxicity only at very high cell densities	Non-sulphated alkaloid a secondary amine, 2-acetyl-9-azabicyclo(4-2-1)non-2-ene	Quebec (Canada): 3.7 µg/L

Table SD8-*I2*: Guideline levels for managing drinking source waters containing cyanobacterial cells applied by different public health organizations (Chorus and Bartram 1999).

Alert level	Density of Cyanobacterial cells	Action
Vigilance	200 cyanobacterial cells/ml	• Non-bloom condition, weekly monitoring
1	2,000 cyanobacterial cells/ml or 1 µg/l chlorophyll-a with a dominance of cyanobacteria	• Weekly cyanobacterial count • Weekly toxin testing to be initiated in drinking water supplies • Issue advisory notice to public
2	100,000 cyanobacterial cells/ml or 50 µg/l chlorophyll-a with a dominance of cyanobacteria	• Weekly cyanobacterial count • Weekly toxin testing in drinking water supplies • More extensive advice to public • Switching to alternative supply should be considered if available

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CHAPTER 9 GENERAL DISCUSSION

This chapter presents a global discussion on the entire results of this Ph.D. research. Within this chapter the novel outcomes of this study will be highlighted and the limitations of the research approach will be discussed. The objectives and the results of this Ph.D. study have been classified into three themes:

1. Occurrence and monitoring: Detection of CB and their associated toxins at the raw water of DWTPs
2. Treatment: Chlorination of CB cells and their associated toxins
3. Management: Development of recommendations for potentially toxic CB monitoring and treatment

9.1 Detection of CB and their associated toxin at the raw water of DWTPs

Results of four consecutive years of monitoring in different DW sources (over the source, at the water intake and inside the DWTP) in southern Quebec (Canada) demonstrate that water authorities and DWTPs have to evaluate the presence of potentially toxic CB and their proliferation over the source in order to evaluate the risk of toxins breakthrough into the treated DW. The frequent monitoring of cyanotoxins provides high-quality documentation of high cyanotoxins concentrations at the water intake of DWTPs (Figure 9.1). Furthermore, CB monitoring results demonstrate the multi-species composition of CB during bloom events in Quebec (Figure 9.2) and the change in dominant species between water bodies and between seasons.

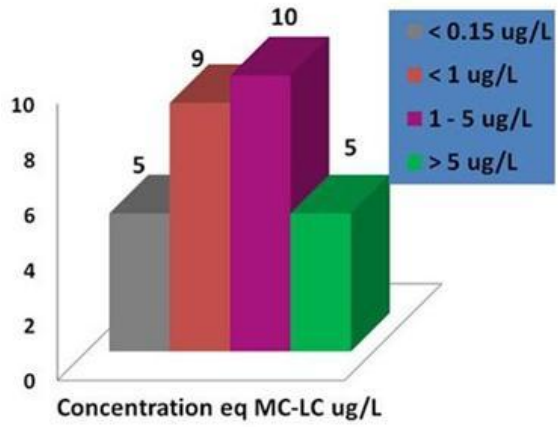

Figure 9.1: Classification of water samples (only one season) from the water intake of the DWTP situated at BM based on the cyanotoxin concentrations (Adapted from McQuaid et al. 2011 and Chapter 4)

The monitoring of CB and their associated toxins is essential. Rapid estimation of CB concentrations, growth phase of CB populations and their associated toxins over the source and the water intake are a necessity for effective management of the risks associated with the presence of cyanotoxins for recreational activities, DW production and water reuse. Low frequency conventional monitoring methods, for example the microscopic enumeration method, cannot provide in situ results and also requires highly qualified personnel. In contrast, fluorescence spectroscopy can provide an *in situ* estimation of CB cell density because fluorescent probes specific for CB monitoring is currently available. Systems evaluated include:

- System 1: YSI *in vivo* PC (YSI 606131), Chl*a* (YSI 606025) fluorescence probes and YSI optic turbidity probe (YSI 606136) installed on an online YSI 6600 V2-4 water quality multi-probe system,
- System 2: TriOS MicroFlu-blue (TriOS)
- System 3: bbe FluroProbe (bbe).

The probe manufactures used two approaches to measure for PC and Chl*a* signatures: (1) a direct measurement by selecting the most selective excitation and emission filters as used by YSI and TriOS, and (2) measuring Chl*a* (and other fluorescence signatures) and using an algorithm to estimate the residual signature of PC, as used by bbe.

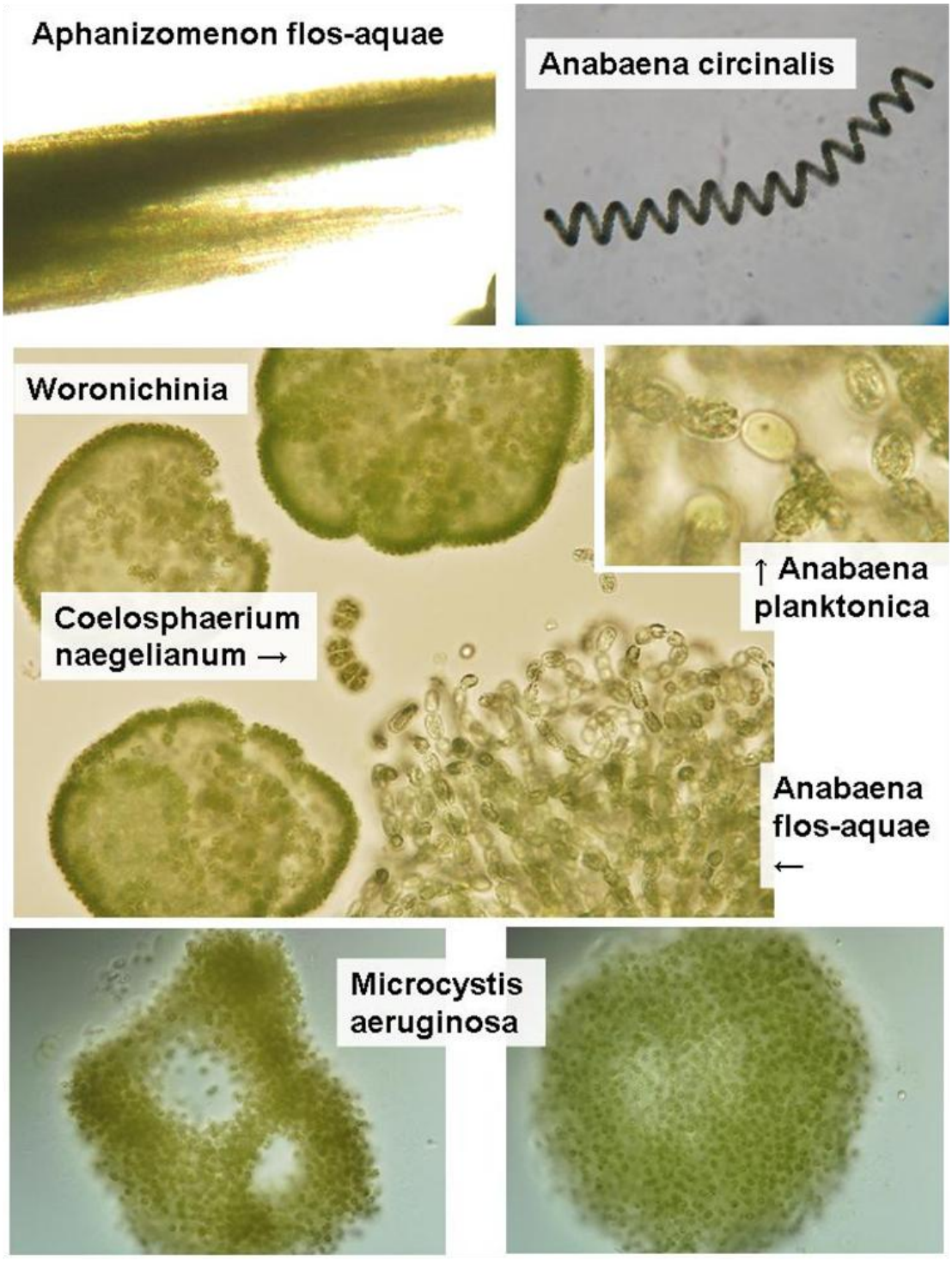

Figure 9.2: Microscopic photo of some species found in one sample from the water body located on Yamaska River basin.

This study focused on identifying the major issues involved with the in situ measurement of CB using the *in vivo* probes. It is possible to classify the interference involved with *in vivo* CB detection and their sources into four categories: (1) the specificity of light source used in the probes and bias caused by Chl*a* of chlorophyta present in water bodies; (2) fluctuations in the PC and Chl*a* concentrations (variations in pigment concentration per cell); (3) abiotic factors, e.g. water turbidity, can interfere with transmission of the excitation wavelength and the cells response to the probe; and (4) presence of phycobilin protein containing Cryptophyta.

The laboratory results on validation of the probes with different CB and algae suspensions showed that the probe responses are highly linear. However, the field validation essays revealed systematic underestimation of the CB biomass and consequently, in some cases, underestimation of alert levels. It has been suggested that the confusion between different phycobilin containing phytoplankton and variation in species present in different locations can be the source of these contradictory results in *in vivo* fluorescence measurement. Indeed, the physiological characteristics of CB and the technical characteristics of the optics of a given probe should be considered. During this Ph.D. research, the probe‟s optic bias, due to simultaneous detection of Chl*a* of Chlorophyta presence in water bodies, was studied in detail and as a result a novel correction factor was proposed. The development of the new probe with the parallel PC and Chl*a* measurement and correction of PC *in vivo* readings, using *in vivo* Chl*a* measurements, is a promising solution to reduce the impact of this bias on the *in vivo* PC readings. However, the performance of the newly developed probe and the parallel PC-Chl*a* correction method has only been tested using laboratory cultured CB in laboratory conditions. The validation of the prototype probe and its correction factor during field measurements is essential.

This dissertation provides novel information regarding the interpretation of data generated by this type of probes and also the need to measure other parameters, such as Chl*a* and water turbidity, simultaneously to improve their interpretation. The presented results highlight the uncertainty in the interpretation of *in vivo* PC measurements in relation to CB cell numbers, CB biovolume, bloom age, alert level thresholds and toxin/odour production. These sources of uncertainty have been a major issue in the application of this technology for management purposes. This dissertation demonstrates that solutions are available for these sources of interference, and moreover, application and interpretation of *in vivo* probe readings (even with biased readings) will be helpful in preventing bloom event calamities like that of BM DWTPs.

9.2 Chlorination of CB cells and their associated toxins

A summary of the available information on oxidation of CB cells and their associated toxins focusing on the chlorination kinetics models can be found in Table 2.6. Also in this table, the domains that required further investigation are highlighted. Table 9.1 demonstrates the contribution of this Ph.D. dissertation to the unknown domain of chlorination of CB cells and their associated toxins. Furthermore, this table will provide the information for comparison of the results achieved under different chlorination conditions.

The efficiency of chlorination on cell lysis depends on the species present, cell densities, the physiological state of the cells (e.g. the growth phase), water matrix and the conditions of oxidation. Based on presented results, the vulnerability of CB cells to chlorination is as follows: *Cylindrospermopsis raciborskii* ~ *Aphanizomenon issatsckenka* > *Anabaena circinalis* > *Microcystis aeruginosa*. Cell damage results match with geosmin and toxins release, however, it is possible to count under a microscope the semi-alive or damaged cells after chlorination. In addition, the auto florescence observations showed chlorinated cells fluorescing even though they were classified as dead cells. Table 9.1 demonstrates a wide variation in the k_{appt} values for chlorine reaction with MC-LR and *Microcystis aeruginosa* cells. This variation is due to raw water quality, CB species, sources of CB (laboratory culture or bloom sample) and sources of toxins used in different experiments.

Furthermore, table 9.1 shows the chlorine reaction with STX is faster than the reaction with C1&2 and GTX2&3. However, when STXs are presented in STXs eq. (e.g. application of ELISA kit for toxin analysis) the estimated reaction rate is representative of chlorine reaction with a mixture of different STXs analogues. This dissertation also presents the first estimation of chlorine demand per CB cells (*Microcystis aeruginosa*) to attain complete lysis (5.6±0.2 $pgCl_2$/cell). The cellular chlorine demand is estimated during chlorination of *Microcystis aeruginosa* in ultrapure water (Chapter 7). Estimation of cellular chlorine demand for the lysis of different CB species requires further investigation.

Table 9.1: Second-order rate constants (k_{appt}) for the reaction chlorine with CB cells and cyanotoxins

Toxin	CB	pH	k_{appt} toxin $M^{-1}s^{-1}$	k_{appt} CB cell $M^{-1}s^{-1}$	Reference
MCs	*Microcystis aeruginosa*	8.3-8.6	-	670-1100 laboratory culture	(Lin et al. 2009)
-	*Microcystis aeruginosa*	8.3-8.6	-	70-590 Taiwan bloom	(Lin et al. 2009)
-	*Microcystis aeruginosa*	7.6	-	43.6	(Ding et al. 2010)
MC-LR	*Microcystis aeruginosa*	7.8-8.3	-	3.78-15.97in Quebec bloom	Chapter 8
MC-LR eq.	*Microcystis aeruginosa*	8.0	14.68-86.86 cell-bound in ultrapure	8.58-27.90 laboratory culture in ultrapure water	Chapter 7
MC-LR	-	8.0	91.5-33 diss.*	-	(Acero et al. 2005)
MC-LR	-	6.8-7.6	242 diss.		(Daly et al. 2007)
MC-LR	*Microcystis aeruginosa*	7.9	10-96 Cell-bound	760 laboratory culture	(Daly et al. 2007)
MC-LR	-	7.6	55.9 diss.	-	(Ding et al. 2010)
MC-LR & MC-LR eq.	*Microcystis aeruginosa*	7-8.5	11.50-234.07 cell-bound + diss.	11.5-1030 laboratory culture	Chapters 7 & 6
MC-LA	-	6.8-7.6	172 diss.	-	(Daly et al. 2007)
MC-LA	-	7.6	89.5	-	(Ding et al. 2010)
MC-RR	-	6.1-8.0	130-34 diss.	-	(Acero et al. 2005)
MC-RR	-	7.6	136 diss.	-	(Ding et al. 2010)
MC-LF	-	7.6	204 diss.	-	(Ding et al. 2010)
MC-LW	-	7.6	3320 diss.	-	(Ding et al. 2010)
MC-YR	-	7.6	94 diss.	-	(Ding et al. 2010)
CYN	-	8.0	490 diss.	-	(Rodriguez, Sordo et al. 2007)
CYN	*Cylindrospermopsis* sp.	8.0	8.2-186.4 cell-bound	3593-4106 laboratory culture	Chapter 6
-	*Aphanizomenon* sp.	8.0	-	3303-4105 laboratory culture	Chapter 6
Anatoxin	-	4.0-12.0	< 1	-	(Rodriguez, Sordo et al. 2007)
-	*Anabaena circinalis*	8.3-8.6	-	1400-3400 laboratory culture	(Lin et al. 2009)
STX	*Anabaena circinalis*	6.8-8.0	293.5-370.5 diss. & cell-bound	1400-3400 laboratory culture	Chapter 5
C1&2 & GTX2&3	*Anabaena circinalis*	8.0	50.9-80.1 diss.	-	Chapter 5
STXs	*Anabaena circinalis*	8.0	51.5-170.9 cell-bound + diss.	1607-2070 laboratory culture	Chapter 6

In order to discuss the results of this research on the integrity of chlorinated cells, a detailed example on CB cell chlorination is presented in Figure 9.3. This figure demonstrates a comprehensive investigation of variations in CB cell integrity due to chlorination. This figure is only a replicate of the chlorination experiments on *Anabaena circinalis* cells; however it is a clear example of the impact of chlorination on cell integrity observed during this Ph.D. research. The addition of chlorine to the cell suspensions significantly altered the proportions of cells classified in the living active cells and the injured/dead cells categories. After a CT of 10 mg.min/L, the proportion of living active cells dropped to a minimum, while the percentage of injured/dead cells rapidly increased. However, less than 20% of cells were actually lysed at this CT. The percentage of lysed cells followed a constant trend and never reached a 100%. These results show that chlorine compromises the integrity of CB cells; therein the chlorinated cells can be classified into three categories of injured/dead cells, dead permeable cells, and lysed cells. The cells fitting to the categories of injured/dead cells and dead permeable cells are detectable under microscope in preserved samples; nonetheless they are cells with membrane damage or metabolically inactive cells.

Measured concentrations of TTHM, HAA6 or HAA9 and NDMA with the highest CT values in natural water spiked with CB cells (below 200,000 cells/mL) were meeting the existing guideline on DBP concentrations in DW. Additionally, these results demonstrate that over 50% of DBP formation in these CB cells suspensions in natural water was caused by the background quality of the natural water and not CB cells. The chlorination of CB cells in ultrapure water with a contact time of 24 hours confirms these results. However, the DBP formation in the water samples from bloom events exceeded the guideline values mainly due to the DOC concentrations from natural organic matter in the water matrix.

The CB cells used were harvested at an early stationary growth phase. EOM release varies widely with the cell growth phase. In the case of CB, while EOM concentrations increase from the onset of growth to early stationary phase, the composition of the EOM varies. During the exponential growth phase, low molecular weight intermediate products such as glycolic and amino acids are dominant, while EOM from senescent cells are typically composed of high molecular weight products such as polysaccharides. Thus, EOM excreted mainly during the exponential growth phase has been shown to be very reactive to chlorine and a precursor to DBP in some cases. Albeit, many of the experiments in which this high reactivity was observed were

conducted in the laboratory using protocols designed to maximize EOM production with elevated concentrations produced using very dense monocultures and extreme chlorination conditions. This dissertation presents the novel data on DBP formation due to direct chlorination of CB cells in conditions close to DWTPs operational situations.

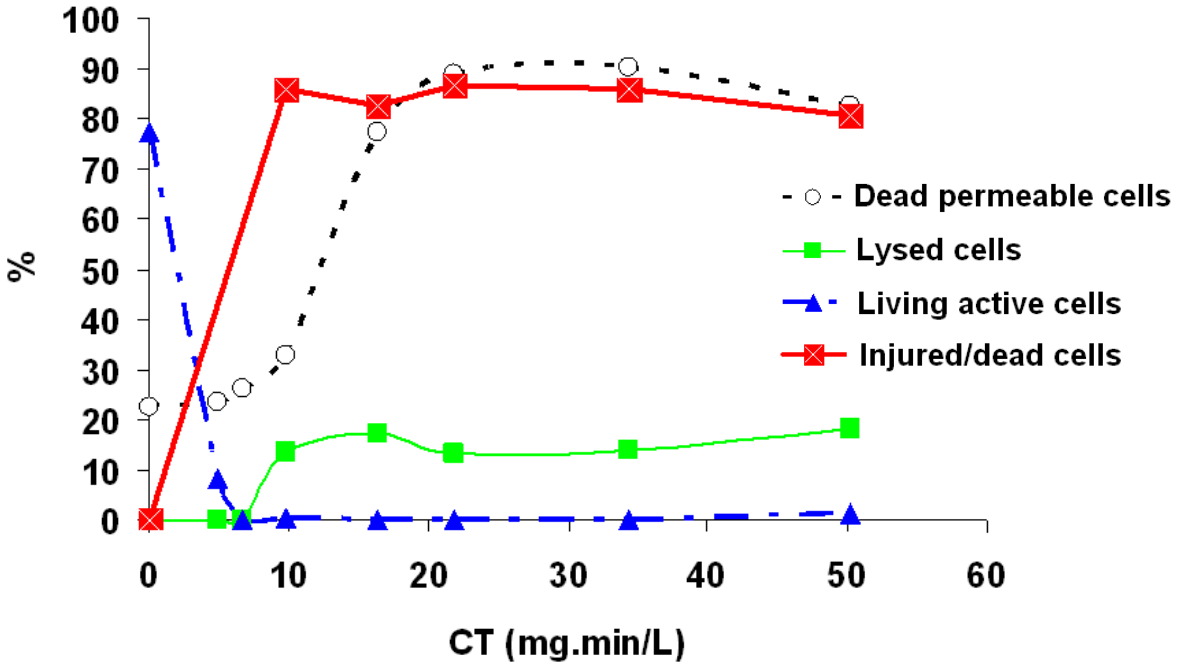

Figure 9.3: Detailed analysis of *Anabaena circinalis* cell integrity due to chlorination

9.3 Development of recommendations for potentially toxic CB monitoring and treatment

The cyanobacterial monitoring in the studied DWTP in Quebec (Canada) documented the presence of toxic cyanobacterial scum inside the DWTP. These results suggest that CB can accumulate and perhaps grow in the clarifier and the filter of the DWTP. The breakthrough of high cell numbers into the water intake of the DWTP intensifies the accumulation process inside the plant. Notably, flow of low cell numbers (even below the 1st Alert level) can also lead to major accumulation of CB inside the DWTP. These results demonstrate the variations in toxin content per cell in both laboratory culture and natural bloom samples, highlighting the

importance of CB monitoring even in low numbers. This study documents the very new information on the speciation of CB cells through different treatment processes.

Clarifiers are designed to agglomerate and settle particulate matter and it is expected that CB cells will be captured and concentrated by this process. The choice of coagulant agents, the CB species and the physiological status of the cells are all key factors in successful settlement of agglomerated cells. The actual accumulation of cells in a clarifier occurs in sludge that is collected in the sludge concentrations section. Moreover, in sludge bed clarifiers, significant amounts of CB cells will accumulate in the sludge bed that serves as a flocculation step. The successful separation of cyanobacterial cells in the clarifier and the sludge retention time inside the clarifier define the level of accumulation of cells and their potential for toxin release. We observed that the majority of *Microcystis* sp. and *Anabaena* sp. cells settled and were removed during the clarification process. The remaining cells of these species were retained by conventional dual media filter; consequently low numbers of these species have been detected in filtered water. *Aphanizomenon* sp. and *Aphanothece* sp. cells are more susceptible to form scums over the clarifier. *Aphanizomenon* sp. has the potential to produce anatoxins (AFSSA-AFSSET, 2006). In the studied DWTP, chlorination has been the only barrier (before DW production) against the potential dissolved anatoxins released by *Aphanizomenon* sp. in clarified water. However, Table 9.1 shows that chlorine is not an efficient oxidant agent for removal of anatoxins. Cyanobacterial cells in the clarified water will lead to accumulation and even breakthrough of potentially toxic cells while the filter run cycle proceeds. Potential risk of toxins release in clarifier and over the filter, the cells‟ breakthrough via the filter, and the impact of the filter backwash on the accumulated cells all need to be quantified. Our results suggest that the accumulation of potentially toxic cyanobacterial cells in any treatment process might lead to breakthrough of cells and their associated toxins to the treated water. The results from chlorination of cells found in filtered water in real world operational conditions were coherent with expected results from chlorination of cells in laboratory conditions.

The studied DWTP draws its raw water from the Missisquoi Bay which is often subject to cyanobacterial proliferations. The concentration of CB in raw water varies widely and is not generally associated with high concentrations of cyanotoxins. However, more frequent measurements during the last four years show that high concentrations of cyanotoxins may be present in raw water at the water intake of the DWTP. In 2010, the total concentration of MCs in

the raw water (at the water intake) of the studied DWTP in Missisquoi Bay reached 118.7 µg/L at one occasion, suggesting that short term elevated toxin concentrations are possible. Very high concentrations of 10,331 µg/L and 171 µg/L of total MCs were detected in the scum observed over the clarifiers and the filters of this facility, respectively. Also, small traces of CYN (0.29 µg/L) were found in the water samples from the scum over the clarifier. The concentration of the total MCs in the sludge bed of the clarifier was 23.92 µg/L. Similar events were observed in 2008 and 2009 inside this DWTP.

The accumulation of toxic cyanobacterial cells inside the DWTP increased concentration of toxins that reached the post-chlorination process. The concentration of the total MCs in the treated DW after chlorination was 2.47 µg/L including 1.74 µg/L MC-LR. The detected total MCs and MC-LR concentration in treated water in 2010 exceeded recommended levels of MCs in DW by Health Canada (1.5 µg/L MC-LR), INSPQ (1.5 µg/L MC-LR), WHO (1 µg/L MC-LR) and International Guidelines (1 µg/L MC-LR). The published literature prior to the conclusion of this study were unanimous about the chlorine efficiency in oxidation of MCs particularly MC-LR. However, the documented results presented in this dissertation provide information closer to the real operational conditions. This information is detailed in results from the studied DWTP (Chapter 8), chlorination of real bloom samples in laboratory conditions (Chapters 6 & 7), and chlorination of 200,000 cell/mL of *Microcystis aeruginosa* in natural water where an initial MC-LR concentration of 20.67 µg/L after a CT of 219 mg.min/L was oxidized to 2.01 µg/L (Chapter 6). Furthermore, the chlorination is not efficient for oxidation of anatoxins and its variants. Anatoxins are one of the toxins of interest in Quebec (Canada).Some of the identified CB species from the last four years of monitoring over DW sources and inside the DWTPs in Quebec have been shown to have a high potential for anatoxin production. These results confirm that the cyanotoxins oxidation kinetics models derived from dissolved toxins in laboratory conditions may not always be valid in natural conditions.

The results from cyanotoxins monitoring inside the DWTP presented in this study only include total toxin concentrations. It is important to understand the fate of cell-bound and dissolved toxins during different treatment processes. Furthermore, for better management of CB inside DWTP it is needed to classify the processes or the phenomenon (hydraulic stress in the pumps or during the filter backwash, etc.) that lead to the release of cell-bound toxins inside the treatment facilities.

Despite the fact that the authorities have followed the levels of cyanotoxin on a regular basis within the studied DWTP and other facilities prone to cyanobacterial blooms, high concentrations of toxins had not been measured in the samples taken. It is hypothesized that the sampling schedules and frequency used did not necessarily coincide with peak periods of toxins. As Australian experiences demonstrate, regular cyanobacterial monitoring has to be conducted at high frequency and it is essential to incorporate the event based monitoring into the cyanobacterial management strategies. The Australian water authorities‟ strategies in management of CB related issues are exemplary. The Australians have successfully adapted intensive *in vivo* CB monitoring, with all the known interferences involved, into their management plans. Furthermore, in bloom season, water bodies are monitored on a daily basis and DWTPs are informed about the CB presence within proximity of their water intakes.

Ultimately, these observations show the importance of (1) targeting maximum periods of proliferation to conduct the risk characterization for CB and their associated toxins, and (2) verifying if this risk can be amplified in the DWTPs. The observations within the studied DWTP and four other sources in Southern Quebec highlight the potential vulnerability of other water intakes and the importance of carrying out more intensive monitoring in sites considered at risk. Very little information is available on the duration and frequency of peak events of CB and cyanotoxins in raw water, their intensity, and the subsequent impact throughout the treatment process.

CHAPTER 10 CONCLUSION AND RECOMMENDATIONS

In order to maintain consistency in this dissertation, the conclusion and recommendations of this research work are presented under the three main themes as of the general discussion.

10.1 Detection of CB and their associated toxin at the raw water of DWTPs

Parallel measurement of PC and Chl*a* is unavoidable for a comprehensive CB management strategy. This is demonstrated by the following results concluded from presented research: CB monitoring results, information on variability in physiological status of the cell particularly PC and Chl*a* cell content and the data showing the phytoplankton dynamics of water bodies. This simultaneous Chl*a* measurement reduces the risk associated with misinterpretation of *in vivo* PC high/low peaks. Furthermore, the proposed correction factor for *in vivo* PC readings using the parallel Chl*a* measurements implemented on the prototype probe can be used to correct commercially available *in vivo* PC probe readings, if simultaneous Chl*a* is available. Further investigation of the new generation of probes and PC-Chl*a* correction factor in DWTP operational conditions is necessary. It is required to study the details of the impact of physiological status of CB cell on the performance of the prototype probes. Moreover, the PC and Chl*a* concentration per cells or per biovolume, toxins concentration per cell or per biovolume, and the cell-bound:dissolved toxins ratio is essential for proper application of the *in vivo* probes.

The probe biases occurred mainly due to the limited wavelengths (maximum 5) measured by the probes with large band-pass. Overall, these probes do not measure all the various fluorescence signals suited for an in situ estimation of cell density, and actual cell-bound and extracellular material. The issues involved with commercially available probes demonstrate the need for modification of *in vivo* measurement techniques. The reported underestimation of fluorescence measurements by *in vivo* probes will risk the accurate estimation of toxin production potentials of CB blooms for management purposes. *In vivo* fluorescence measurements require a frequent validation with more solid methods for better speciation and biomass correlation.

Even though correction factors and modified procedures have been recently developed for optimum application of *in vivo* probes, it is not possible to use these probes to evaluate the stage

of growth of the cyanobacterial population. The accumulations of toxic CB cells in the studied DWTP proved that it is not only necessary to conduct an *in vivo* monitoring, but that it is more important to monitor the growth of the cyanobacterial population over the source, at proximity of the water intake, and at the water profile over the intake. Application of suitable fluorescence marker signals that detect the presence of toxic cyanobacterial population from early growth phase is essential for proactive management plans. Furthermore, the Australian water authorities and experts have highlighted that tracking of CB growth rate is an essential factor for the appropriate monitoring and management of toxic CB bloom events. Thus, the question of online monitoring of the growth rate of CB and their toxic production needs to be answered. The vital consideration in operations monitoring is the possible health consequences of missing an early diagnosis of a CB related problem. The *in vivo* probes do not provide the opportunity to observe the changes in CB cells fluorescence during their growth, as they only detect the pigment signature of the CB.

New advances in Fluorescence Excitation-Emission Matrix (EEM) technology have direct application for CB blooms monitoring. This is because, in addition to specific pigments, CB also exhibit other fluorescent characteristics including aromatic amino acids and quinoid groups that are species specific and fluoresce in the short UV wavelength range. It has been demonstrated that the age of the cyanobacterial population influences the fluorescence spectrum of its extracellular and intracellular substances. Hence, it is possible to monitor the growth of the cyanobacterial population to prevent the CB breakthrough into the DWTPs and their accumulation using EEM. However, further studies are required to develop this idea.

This study demonstrates that even though biases are involved with *in vivo* measurements and that development of novel technologies are required to overcome the actual challenges, application of existing *in vivo* probes will help to prevent the accumulation of potential toxic CB cells inside the DWTPs and toxins breakthrough into the DW. Furthermore, these results show that with all biases involved with *in vivo* probe readings it would have been possible to prevent the documented treatment disturbance had the operation of the DWTP located on MB been equipped with a probe and a threshold adapted to the probe readings.

10.2 Chlorination of CB cells and their associated toxins

Our results show that inactivation of CB cells with chlorine is a feasible option. However, the compromised cells remain in water samples and preservation of chlorinated samples with Lugol‟s iodine fixes the cell form (detectable under the microscope) even if they are metabolically inactive. Consequently, attention should be given to provide adequate CT to guarantee the efficient oxidation of the released recalcitrant cyanotoxin analogues. These data provide novel information for DWTP to establish given that the toxin release or oxidation rate is the limiting step in the toxin oxidation. Though, in the case of CB species producing T&O compounds, e.g. geosmin and MIB, utilities might still face the challenge of odor due to released compounds. Results from this study suggest that pre-chlorination of natural water containing CB cell numbers ≤200,000 cells/mL for different treatment purposes is a feasible practice. The variations in chlorine reaction with CB cells and the cyanotoxins (Table 10.1) demonstrate the important effect of raw water quality, CB species, source of CB used in the experiment, and the source of toxins on chlorination efficiency. DWTPs operators and water authorities have to take into consideration these sources of variation prior to integration of the published data on chlorination of CB cells and their associated toxins in operational situations.

Careful interpretation and application of the published second-order constant rate values for operational purposes is essential, as misinterpretation of k_{appt} values might lead to insufficient oxidation of toxins. It is needed to describe the application of the Table 10.1 and the novel outcomes of this dissertation on kinetics of chlorination of cyanotoxins in different conditions in a format applicable to DWTP operational conditions. The chlorination condition and the applied CTs are mainly designed to achieve standard removal of microbial infection e.g. *Giardia*. USEPA guideline suggests that chlorine CT values of 75 mg.min/L at pH 7.5 and 91 mg.min/L at pH 8 (Initial Cl_2 concentration = 2 mg/L which is close to what is practiced in the studied DWTP in Quebec, 20^{o}C, $T_{10}/T = 0.8$) for 3 log inactivation of *Giardia* cysts by free chlorine is required (United States Environmental Protection Agency (USEPA) 2003). Table 10.1 and the presented second-order kinetics help the operators to evaluate if the chlorination applied in their facility for the removal of *Giardia* cysts would be sufficient for oxidation of cyanotoxins. For this purpose Figures 10.1, 10.2 and 10.3 are presented in this section.

A very common misinterpretation of k_{appt} values is the generalisation of the kinetic models of the most known toxin analogues to the less known analogues, while some analogs are more recalcitrant. STX is the most well known of the STXs analogues and based on the novel chlorination kinetics presented in this dissertation, STX reaction with chlorine is faster than other STXs analogues. Figure 10.1 presents the required chlorine CT for removal of STX and GTX3 calculated using the k_{appt} values. This figure demonstrates that with enough CT for removal of 3 log of *Giardia*, 3 log removal of STX will be achieved easily; however only <2 log removal of GTX3 will be achieved. All STXs analogues are very toxic (LD_{50} = 10 to 30) and their removal below the recommended threshold is essential.

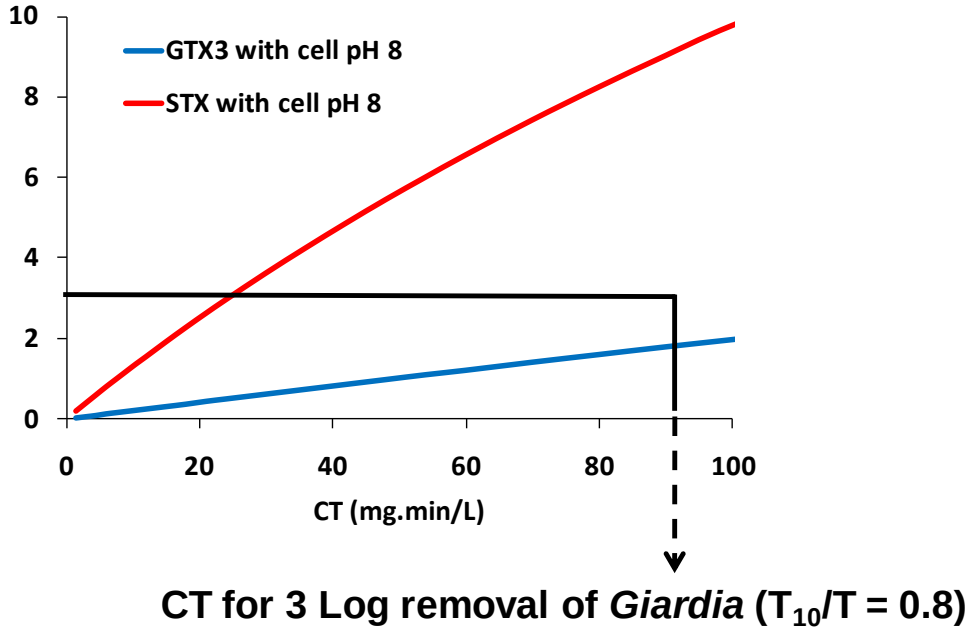

Figure 10.1: Log removal of two STXs analogues (cell-bound STXs released from compromised cells) with different chlorine CT values.

The first published kinetic values for chlorination of cyanotoxins (mainly MC-LR) were based on experiments using dissolved toxins in the absence of cells. These experiments are important to demonstrate that chlorine is capable of oxidizing the toxins. Though, in operational conditions, toxins originate from CB cells and the chlorination occurs in the presence of cell and cellular materials. It is important to verify if the laboratory based kinetic models using standard toxins are compatible with the operational conditions. Figure 10.2 presents the chlorination of cell-

bound STX released from compromised *Anabaena circinalis* cells (in presence of cells) and standard STX material in the absence of cellular materials. For STX, the rate of chlorine reaction in both conditions is quasi constant and with 3 log removal of Giardia, more than 6 log removal of STX is feasible (Figure 10.2). However, Figure 10.3 shows that unlike STX, chlorination of MC-LR from different origins is not following constant kinetics. Based on the apparent constant rate estimated for chlorination of dissolved MC-LR the CT of 75 mg.min/L at pH 7.5 is sufficient for 3 log removal of the toxin. Nevertheless, the apparent constant rate estimated for chlorination of cell-bound MC-LR shows that under this CT 3 log removal of the toxin is not feasible (Figure 10.3).

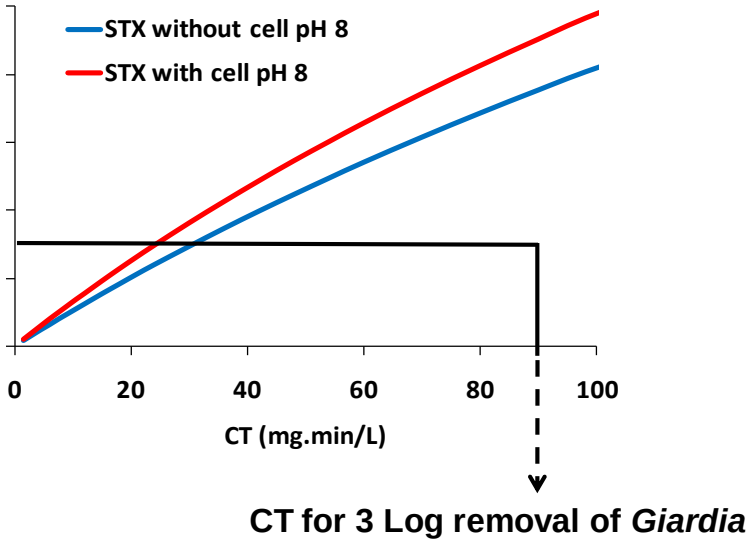

Figure 10.2: Log removal of STX from different origins with different chlorine CT values.

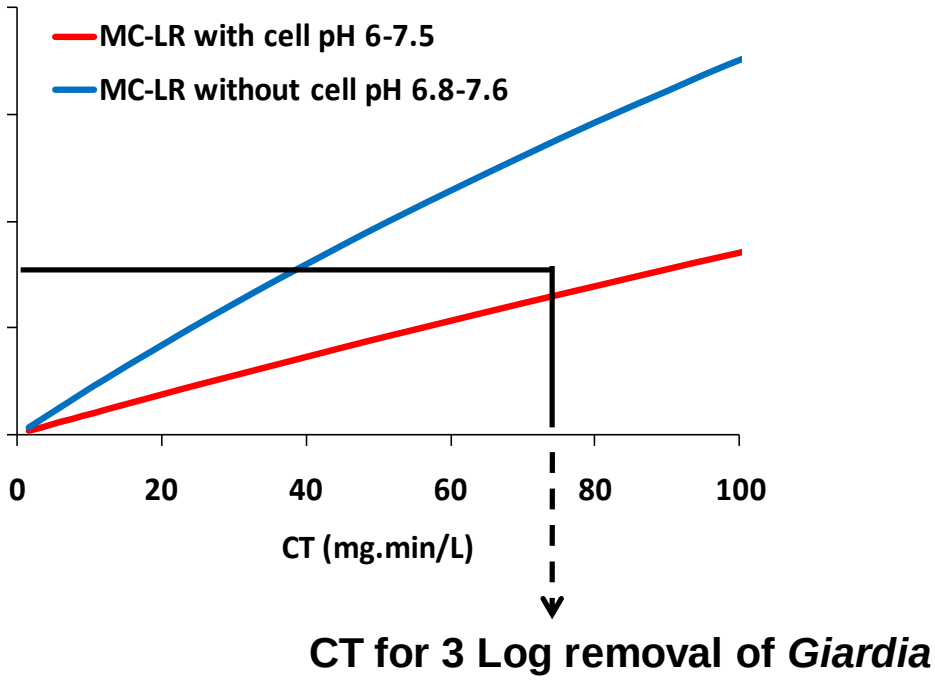

Figure 10.3: Log removal of MC-LR from different origins with different chlorine CT values

This dissertation concludes by stating that the type of toxins found in the water body, and not the CB cells, influence the oxidation options. However, the biovolume of present CB species might influence their breakthrough inside treatment facilities. In the case of the Australian treatment facilities, chlorine is a sufficient option as chlorine is an efficient oxidant agent for removal of toxins found in Australian CB bloom (MCs, STXs and CYN). While in the province of Quebec (Canada), the simultaneous presence of MCs, CYN and anatoxins complicate the oxidation options as chlorine is not effective in oxidation of anatoxins under the CT values used in DWTPs.

Removal of CB cells and their associated toxins in pre-treatment by pre-chlorination is possible. Pre-chlorination removes cells and their associated toxin; it also helps to reduce the negative impact of cell accumulation on other treatment processes (filters clogging, waste of water during more frequent backwash). The treatment efficiency is directly related to the cell number and the toxin concentration, highlighting the importance of intensive monitoring for best-practice treatment purposes. However, the application of pre-chlorination is limited by the DBP formation potential of raw water. This dissertation shows that in direct chlorination of raw water

containing CB cells, the natural organic matter (DOC background) is the key factor defining DBP formation, not the CB cells related materials. Our results show that it is possible to achieve conflicting water quality goals (effective toxin oxidation while respecting DBP guidelines) using chlorination conditions that would be experienced in a DWTP.

10.3 Development of recommendations for potentially toxic CB monitoring and treatment

This dissertation provides solid evidence on the accumulation of potentially toxic CB cells inside DWTPs (Figure 10.4) and the breakthrough of cyanotoxin into DW. It was believed that major CB related issues in Quebec (Canada) are only restricted to the summer heat wave periods. Noteworthily, the observations of recent years (CB bloom events from May till November) and the documented events of toxic CB breakthrough into DWTPs (Figure 10.4) underline the urgent need for an all-inclusive management strategy focusing on site specific challenges. The success of Australian management of CB related issues are due to (1) the intensive *in vivo* CB monitoring programs (using fluorescence probes) followed by sampling to validate the *in vivo* methods, and (2) the application of practical management guidelines adapted to site specific needs. The conclusions of this dissertation on management issues and relevant proposals to the situation in Quebec are summarized in two parts:

Figure 10.4: Accumulation of potentially toxic CB cells inside the studied DWTP in Quebec (Canada)

A first conclusion emphasizes the need to know the magnitude of the CB related problems in the regions of concern using the analysis of historical data and acquisition of new data in water bodies and inside the DWTPs. To acquire these new data, the use of intensive *in vivo* measurement methods in parallel with conventional methods is essential. The *in vivo* methods have the great advantage of providing in situ data with high frequencies (e.g. every 30 minutes).

This dissertation provides detailed information on the experiences of the Australian DWTPs‟ operators (e.g. temporary closure of the plants for filter cleaning and treatment adjustment) facing the potentially toxic CB issues in their water sources. The presented observations

demonstrate that it is essential to plan intensive *in vivo* monitoring of CB blooms and establish preventive procedures (e.g. change the water intake depth, treatment adjustment or even temporary closure of the water intake) based on the *in vivo* measurements. This Ph.D. research has shown that major sources of interferences should be accounted for when using *in vivo* fluorescence measurements. Technical visits to the Australian water authorities also revealed that they are aware of the bias involved with the *in vivo* probes readings. As presented in this document, the interpretation of *in vivo* data is a key factor prior to their application in management guidelines. These *in vivo* tools provide the water authorities with the information about the tendency of the CB population in water bodies in a frequency that no other method is capable of.

The treatment adjustments require the proper knowledge of the fate of CB and their associated toxins during different treatment processes. This research or treatise provides novel information about accumulation of CB cells inside the plants and the toxic breakthrough to the DW water, yet there is not enough information available to understand the details of these phenomena. Furthermore, it is needed to understand if the observed accumulation and breakthrough are separate incidences or whether they are regular issues occurring during bloom season. In addition, a comprehensive monitoring program (from source to consumers‟ tap) to ensure the protection of public health is strongly recommended. It is necessary to conduct a systematic study (using *in vivo* probes and regular sampling) in order to monitor the presence of potentially toxic CB inside the DWTPs in Quebec. In accordance with the Australian information on CB monitoring and the data presented in this dissertation, it is essential to monitor the presence of potentially toxic CB at several phases of the treatment process: at the water intake, during addition of reactive and PAC, on the clarifier and the sludge of the sedimentation tank, on the filter, in the filtered water itself and in the post oxidation water. Subsequently, it is necessary to adapt the DW guidelines to the new finding based on the site specific needs.

Current international and Quebec guidelines on CB monitoring and management do not fully take into account the potential for toxic CB accumulation and subsequent cyanotoxins release in water treatment processes. The potentially toxic CB challenge for DW production has to be studied separately from other water applications. Sustained low levels of CB cells or localized bloom events might not be a source of concern for a water body. However, they could represent

significant treatment challenges for a DWTP if the local bloom is found at proximity of the water intake.

Another key finding is the requirement to take into account the needs of the individuals and the organizations affected by the CB. It appears necessary to measure their satisfaction with actual management plans and the current threshold values. Built on meetings with experts from Australia, we proposed and were granted a project to investigate the state of awareness of the existence of current intervention guides in Quebec and whether these guides meet the needs of water authorities in Quebec facing the CB bloom events and their associated toxins. The adaptation of scientific and technological solutions and thresholds with local needs for a practical guide is a key factor in the prevention of documented incidents.

This dissertation recommends surveying the applicability of the actual Quebec guidelines using a pointed questionnaire derived from previous surveying experience of Australian water authorities The findings of this survey will help to modify CB management guidelines using the (1) novel knowledge and engineering expertise on detection and treatment of CB and their associated toxins, and (2) the site specific problems of Quebec water authorities.

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APPENDIX A — Microbiology of CB and bloom formation

The cyanobacteria are the largest and most diverse group of photosynthetic bacteria. They have chlorophyll-a and carry out oxygenic photosynthesis. Photosynthetic pigments and electron transport chain components are located in thylakoid membranes lined with particles called phycobilisomes. These contain phycobilin pigments, particularly phycocyanin and transport energy to photosystem. Phycocyanin (maximum absorption 620 to 640 nm) is often called accessory pigments because of their role in photosynthesis (Prescott et al., 2005). Although chlorophyll cannot absorb light energy effectively in the blue green light through yellow range (about 470 to 630 nm), accessory pigment do absorb light in this region and transfer the trapped energy to chlorophyll (Prescott et al., 2005).

Cyanobacteria also vary greatly in shape and appearance. They range in diameter from about 1 to 10 μm and may be unicellular, exist as colonies or many shapes, or form filaments called trichomes (Prescott et al., 2005). The accumulation of many excessively buoyant cyanobacterial cells or colonies (scum) at the surface of water bodies is called a bloom event or proliferation. In extreme cases, such agglomeration may become very dense and even acquire a gelatinous consistency, and sometimes even look like blue-green paint or jelly (The Cooperative Research Centre for Water Quality and Treatment (Australia), 2004). The nature of cyanobacterial proliferation is very dynamic and the bloom event will be followed by a dying-off phase (AFSSA-AFSSET, 2006).

One of the main contributing factors in the formation of cyanobacterial bloom is nutrients, particularly nitrogen, phosphorus and iron, which control the biomass. With the increasing nutrient pollution of freshwater ecosystems, particularly high phosphorus levels (over 25 μg/L), many reservoirs, including those used as DW resources, suffer from extensive cyanobacterial blooms in the summer (Gregor et al., 2007;The Cooperative Research Centre for Water Quality and Treatment (Australia), 2004). Significant changes in the native benthic community might cause the loss of nitrogen forms for key phytoplankton species and the phytoplankton are consequently replaced with CB capable of nitrogen fixation (Hoffman, Rattner et al., 1995). Low nitrogen-phosphorus ratios (less than 29:1) favour the development of cyanobacterial bloom (The Cooperative Research Centre for Water Quality and Treatment (Australia), 2004). In

Québec, the main source of phosphorus is agricultural activities through the whole year, which has a direct impact on cyanobacterial blooms.

Watershed slopes, lake or water reservoir depths, precipitation, land use, prevailing winds, air and water temperature are other factors affecting bloom formation. Low turbulence leading to the reduction of turbidity to moderate levels, and long residence time as well as increased light intensity also all favour cyanobacterial dominance in water bodies (Hudnell, 2006; The Cooperative Research Centre for Water Quality and Treatment (Australia), 2004).

Furthermore, CB have a better ability to avoid predation than do other micro-algae. This ability is related to their particular cellular structure and their ability to synthesize toxins. Many types of CB are organized in the form of filaments or trichomes (e.g. Anabaena sp., Planktothrix sp., Oscillatoria sp.), which can be assembled into bundles or beams (e.g. Aphanizomenon sp.), or in the form of colonies (e.g. Microcystis sp., Aphanothece sp.). This special organization makes their ingestion more difficult for most grazing zooplanktons. However, if such zooplanktons are present prior to the formation of filaments or colonies to a size that protects CB from predation; they can still control the cyanobacterial population (AFSSA-AFSSET, 2006).

APPENDIX B — Potentially toxic species of cyanobacteria and their detected toxins

Non-exhaustive list of potentially toxic species of cyanobacteria and their associated toxins (N.I: Toxins were present but was not identified) (adapted from: AFSSA-AFSSET, 2006)

Toxic cyanobacteria	Cyanotoxins
Anabaena affinis	N.I.
Anabaena circinalis	Anatoxin-a, Saxitoxins, Microcystins
Anabaena flos-aquae	Anatoxins (-a, -a(s), -b, -b(s), -c, -d), Microcystins
Anabaena hassallii	N.I.
Anabaena lemmerman	Microcystins, Anatoxin-a(s)
Anabaena planktonica	Anatoxin-a
Anabaena spiroides	Anatoxin-a, Microcystins
Anabaena torulosa	N.I.
Anabaena variabilis	N.I.
Anabaena sp.	Anatoxin-a
Anabaenopsis milleri	Microcystins
Aphanizomenon flos-aquae	Anatoxin-a, Saxitoxins
Aphanizomenon ovalisporum	Cylindrospermopsin
Aphanizomenon sp.	Anatoxin-a
Coelosphaerium naegelianum	Hépatotoxin
Cylindrospermopsis raciborskii	Cylindrospermopsin, Saxitoxins
Cylindrospermum sp.	Anatoxin-a
Fischerella epiphytica	N.I.
Gloeotrichia echinulata	N.I.
Gloeotrichia pisum	N.I.
Hapalosiphon hibernicus	Microcystins
Lyngbya birgei	N.I.
Lyngbya gracilis	Debromoaplysiatoxin
Lyngbya major	N.I.
Lyngbya majuscula	Lyngbyatoxin-a
Lyngbya wollei	Saxitoxins
Microcoleus lyngbyaceus	N.I.
Microcystis aeruginosa	Microcystins
Microcystis botrys	Microcystins
Microcystis farlowian	Ichtyotoxin
Microcystis flos-aquae	Microcystins
Microcystis panniformis	Microcystins
Microcystis toxica	N.I.
Microcystis viridis	Microcystins, Microviridin
Microcystis wesenbergii	Microcystins
Microcystis sp.	Anatoxin-a
Nodularia spumigena	Nodularins
Nostoc paludosum	N.I.
Nostoc rivulare	N.I.
Nostoc sp.	Microcystins
Oscillatoria formosa	Homoanatoxin-a
Oscillatoria lacustris	N.I.
Oscillatoria limosa	Microcystins
Oscillatoria tenuis	Microcystins
Oscillatoria nigroviridis	Oscillatoxin-a
Oscillatoria sp.	Anatoxin-a
Phormidium favosum	Anatoxin-a
Planktothrix agardhii	Microcystins
Planktothrix mougeotii	Microcystins
Planktothrix rubescens	Microcystins
Planktothrix sp.	Anatoxin-a
Pseudanabaena sp.	Neurotoxin
Raphidiopsis sp.	Cylindrospermopsin
Schizothrix calcicola	Aplysiatoxins
Scytonema hofmanni	Scytophycins a et b
Scytonema pseudohofmanni	Scytophycins a et b
Spirulina subsalsa	N.I.
Symploca hydnoides	N.I.
Symploca muscorum	Aplysiatoxin
Synechococcus sp.	N.I.
Trichodesmium erythraeum	Neurotoxin
Umezakia natans	Cylindrospermopsin
Woronichinia naegeliana anciennement Gomphosphaeria naegelianum	Anatoxin-a

APPENDIX C — Chemical structures of different cyanotoxins

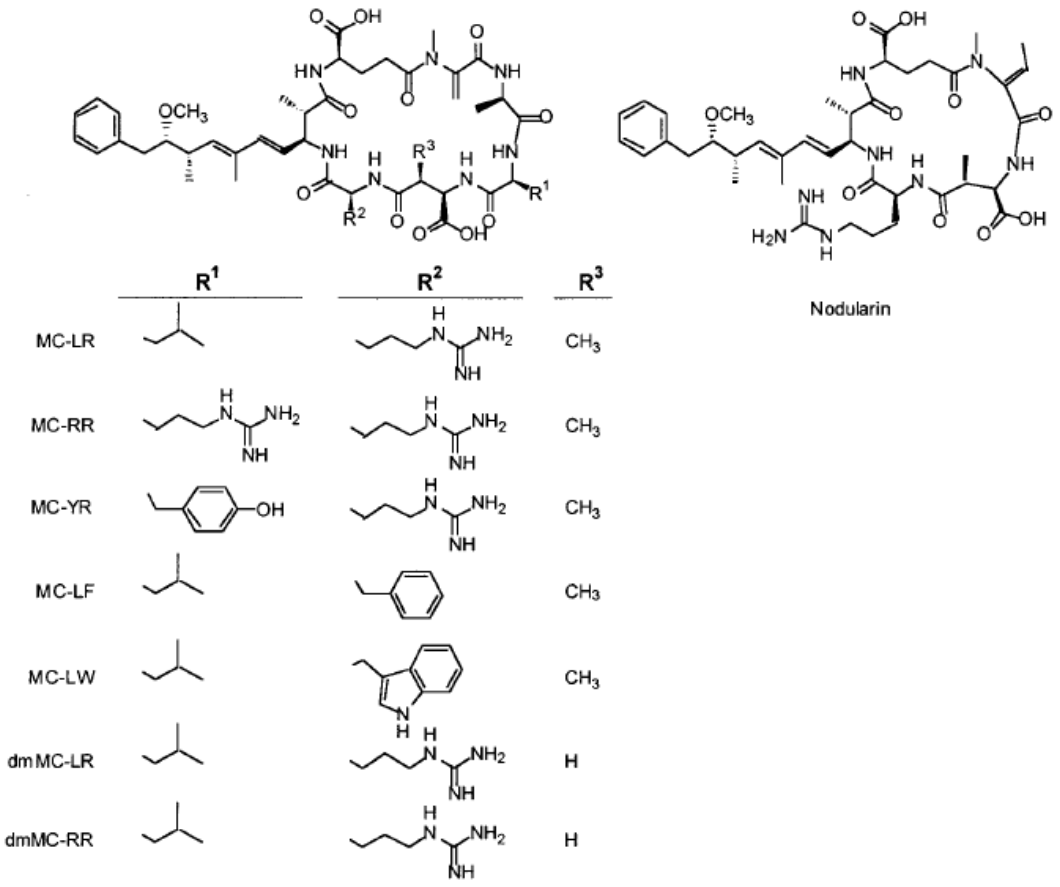


Figure C.1: Chemical structures of different variants of MCs, 3-desmethylmicrocystins (dmMC) and nodularin (adapted from Fischer et al., 2001)

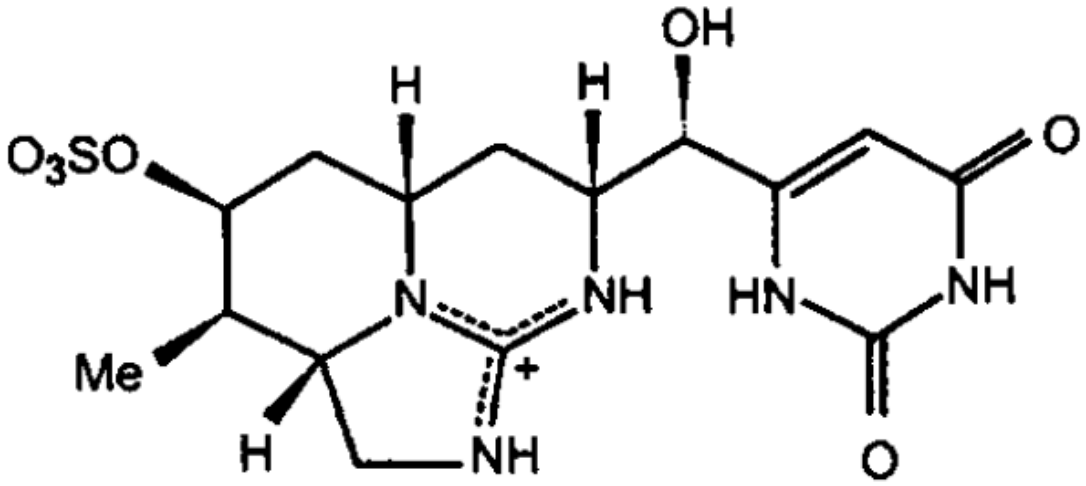


Figure C.2: Chemical structure of CYN (adapted from Chorus & Bartram, 1999)

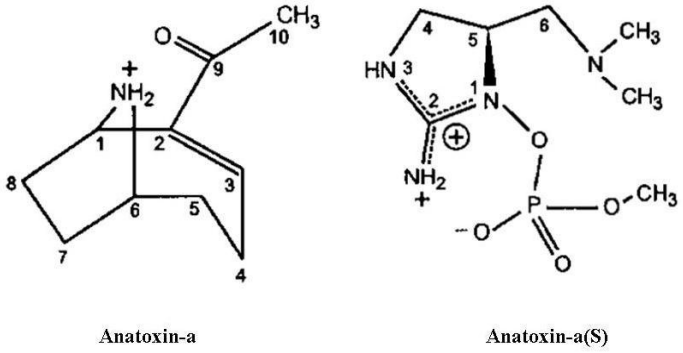


Figure C.3: Chemical structure of Anatoxin-a and a(S) (Adapted from Chorus & Bartram, 1999)

Toxin	R1	R2	R3	R5
R4 = $CONH_2$ (carbamate toxins)				
STX	H	H	H	OH
neoSTX	OH	H	H	OH
GTX1	OH	H	OSO_3^-	OH
GTX2	H	H	OSO_3^-	OH
GTX3	H	OSO_3^-	H	OH
GTX4	OH	OSO_3^-	H	OH
R4 = $CONHSO_3^-$ (n-sulfocarbamoyl (sulfamate) toxins)				
GTX5 (B1)	H	H	H	OH
GTX6 (B2)	OH	H	H	OH
C1	H	H	OSO_3^-	OH
C2	H	OSO_3^-	H	OH
C3	OH	H	OSO_3^-	OH
C4	OH	OSO_3^-	H	OH

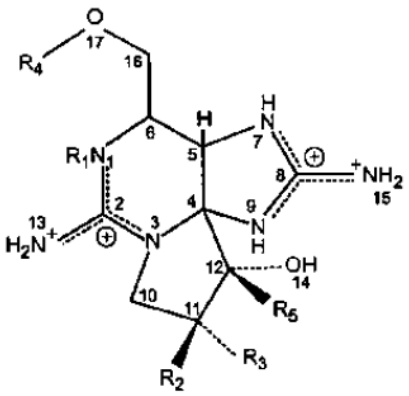
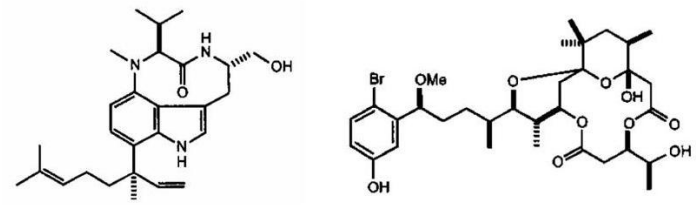


Figure C.4: Chemical structure of STXs (Adapted from Chorus & Bartram, 1999; Nicholson et al., 2007)



Lyngbyatoxin-a

Debromoaplysiatoxin

Figure C.5: Chemical stricture of debromoaplysiatoxin and lyngbyatoxin-a (Adapted from Chorus & Bartram, 1999)

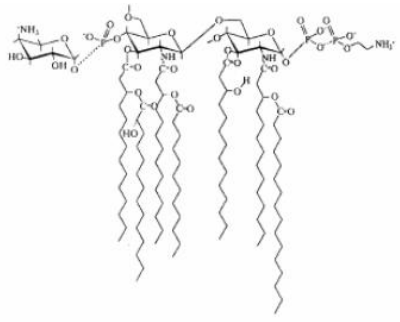


Figure C.6: Chemical structure of LPS (Adapted from Lavoie et al., 2007)

APPENDIX D — Occurrence of CB and their associated toxins and their health impact

Table D.1: Examples of human health problems with CB and their associated toxins (Adapted from Höger, 2003; Svrcek & Smith, 2004)

Location	Year	Cyanobacteria	Cyanotoxin	Human health outcome
Charleston, West Virginia, Ohio and Potomac River, US	1930-1931	*Microcystis* sp.	Unknown	Gastrointestinal disease
Saskatchewan, Canada	1959	*Microcystis* sp., *Anabaena circinalis*	-	Gastrointestinal disease, nervous system affected
Harare, Zimbabwe	1960-1965	*Microcystis aeruginosa*	Unknown	Gastrointestinal disease
Allegheny Country, Pennsylvania, Sewickley reservoir	1975	*Schizothrix*, *Lyngbya*, *Plectinema*, *Phormidium*	-	Gastrointestinal disease
Sewickley, Pa., US	1976	*Schizothrix calcicola*, *Plectonema*, *Phormidium*, *Lyngbya*	Unknown	Gastrointestinal disease
Solomon Dam Palm Island, Australia	1979	*Cylindrospermopsis*	-	Gastrointestinal disease, liver damage, kidney damage, intestinal damage
Malpas Dam, Armidale, Australia	1981	*Microcystis* sp.	-	liver damage
Armidale, Australia	1983	*Microcystis aeruginosa*	Unknown	liver damage, constipation and bloody diarrhea, kidney damange
Palm Island, Australia	1983	*Cylindrospermopsin Raciborskii*	CYN	hepatoenteritis
Itaparica Dam, Brazil	1988	*Anabaena* sp., *Microcystis* sp.	-	Gastrointestinal disease, 88 deaths
Rudyard reservoir, Staffordshire, UK	1989	*Microcystis aeruginosa*	MC-LR	Vomiting, dermatitis, pneumonia, diarrhea Gastrointestinal disease, nervous system affected
South-east, China	1972-1990	*Microcystis* sp.	-	Primary liver cancer
Towns at river Murray, Australia	1992	*Anabaena circinalis*	-	Multiple symptoms
Itaparica Dam, Brazil	1993	*Anabaena* sp., *Microcystis* sp.	Unknown	88 deaths, gastroenteritis
Scania, Sweden	1994	*Planktothrix agardhii*	-	Gastrointestinal disease
Nandong District, Jiangsu Province, Nanhui/Shanghai, Fusui, China	1994-1995	*M. aeruginosa*, *Plankothrix agardhii*, *Anabaena* sp., *Lyngbya* sp.	MC	primary liver cancer correlation
Caruaru, Brazil	1996	*Microcystis Anabaena, Cylindrosopermopsis* sp., *Aphanizomenon* sp., *Oscillatoria* sp.	MC, CYN	liver damage, nervous system affected, Gastrointestinal disease, after hemodialysis by 98% of patients: 76 deaths

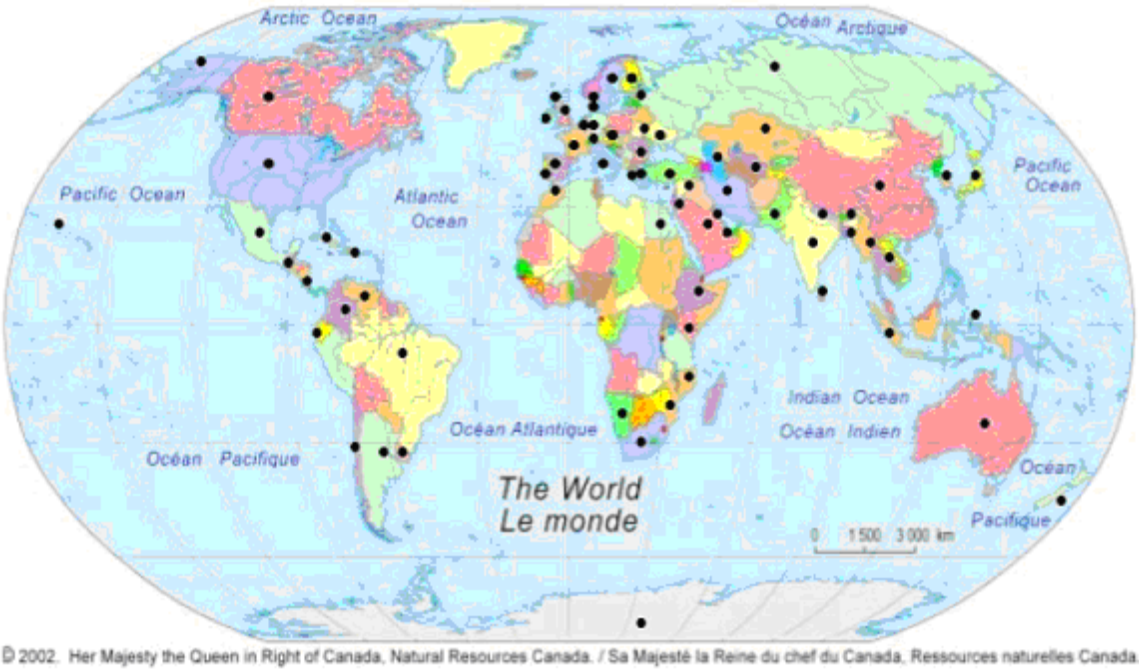
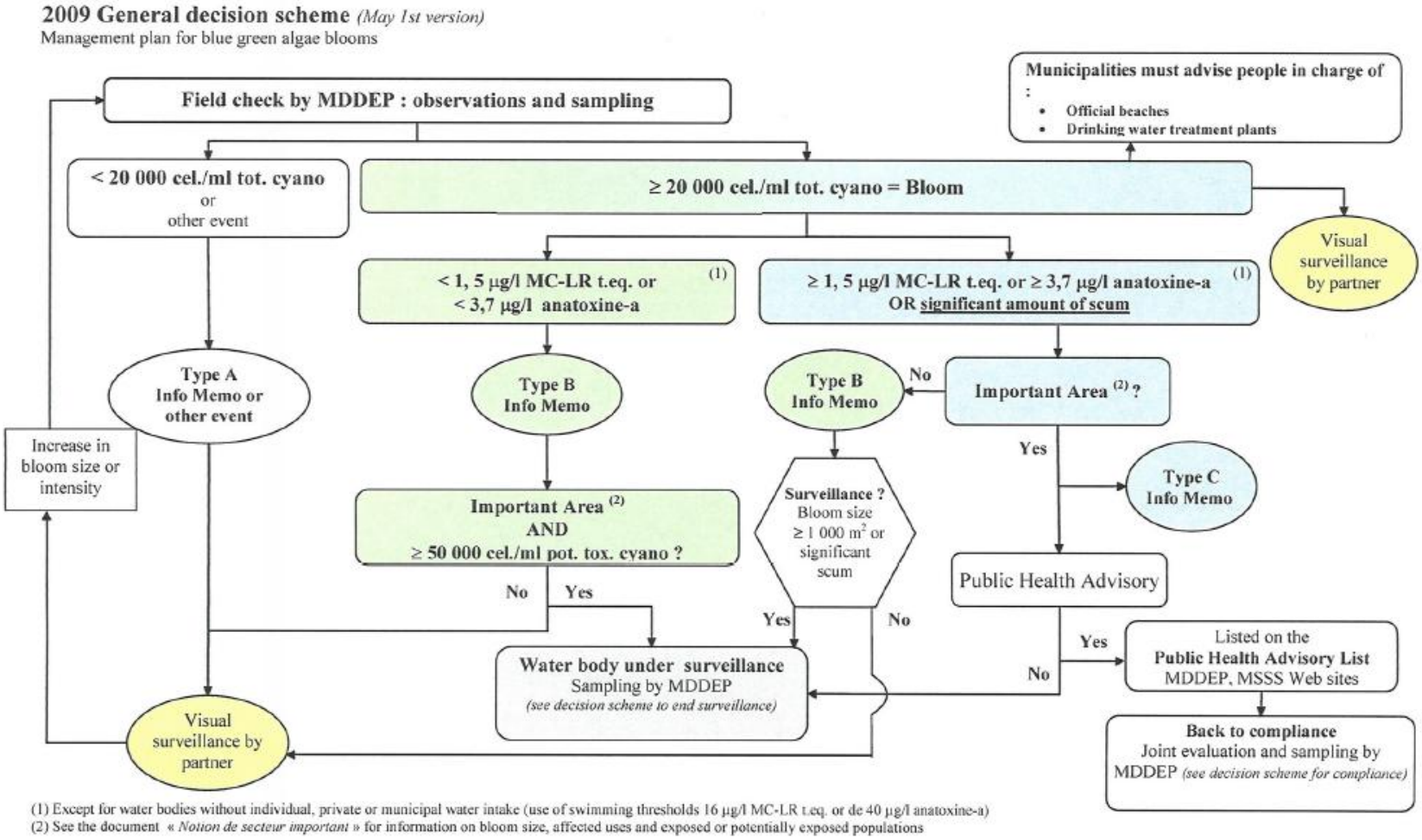


Figure D.1: Countries exhibiting one or more documented harmful cyanobacterial events (Adapted from: Hudnell, 2006)

APPENDIX E — Decision support flow chart for cyanobacterial management in Québec (Canada)

This flow chart has been adapted from Bowling, 2010.



(1) Except for water bodies without individual, private or municipal water intake (use of swimming thresholds 16 µg/l MC-LR t.eq. or de 40 µg/l anatoxine-a)
(2) See the document « *Notion de secteur important* » for information on bloom size, affected uses and exposed or potentially exposed populations

APPENDIX F — FQRNT scholarship for Ph.D. student international internship on cyanobacteria: report on Australian visit

The effects of global climate change appear to enhance the development of the potentially toxic cyanobacterial blooms in surface water sources across the world (Paul, 2008; Dale et al. 2006; Barbeau et al. 2009). To manage cyanobacteria (CB) related issues the Quebec government is conducting a 10 year intervention plan. This plan includes financial support for partnership research programmes between leading Quebec, Canadian and international research groups. The financial support is provided via les Fonds Québécois de la recherche sur la nature et les technologie/Quebec Fund for the Research on Nature and Technologies (FQRNT) for research projects and internships. A project grouping the drinking water industrial chair of École Polytechnique de Montreal/Polytechnique School of Montreal (ÉPM), the limnology research team of Université de Québec à Montreal/University of Quebec at Montreal (UQÀM) and the Australian Water Quality Centre (AWQC) have been formed in 2008 to support a comprehensive research on CB issues. An essential part of this project is to document Australian water authorities‟ experiences in dealing with CB related problems. Therefore, a scholarship was offered by FQRNT to the Ph.D. student Arash Zamyadi to realize a series of industrial visits in Australia. The objectives of this visit (March-July 2010) were (1) to collect and analyze the information from water authorities and research centres across Australia working on monitoring and removal of CB and their associated toxins, and (2) to calibrate the *in vivo* phycocyanin (PC) probes for the in situ CB detection and definition of correction factors for probes readings.

Six Australian water authorities/industries and four Australian research centres dealing with CB related issues were visited. To document the results of these visits a questionnaire was prepared and completed during these visits (Supporting Information (SI) 8.A). Name and affiliation of interviewed individuals are listed in SI 8.B. The author of this report would like to acknowledge the fine collaboration of visited water authorities.

F.1 Sydney Catchment Authority (SCA)

The Sydney Catchment Authority (SCA) is responsible for the management of five main catchments that provide drinking water to Sydney and surrounding areas (SI 8.C Figure SI 8.C.1): Warragamba, Shoalhaven, Woronora, Upper Nepean, and Blue Mountains. These catchments cover an area of more than 16,000 Km^2. More than four million people, or about

60% of the NSW population, consume water supplied by these catchments. About 3700 Km^2 of land within these catchments are known as „Special Areas". These are large areas of mostly unspoilt bushland surrounding the reservoirs that provide drinking water for Sydney, the Blue Mountains and the Illawarra. Special Areas are vital to protect the drinking water because they help filter out nutrients and other substances before the water reaches the reservoirs. The Special Areas are managed by the SCA and the Department of Environment and Climate Change (Government of NSW). Public access to Special Areas is restricted to protect water quality.

The SCA has adopted Australian recognised standards and guidelines for the CB related water quality variables in each part of the water supply network (Sydney Catchment Authority, 2007; Sydney Catchment Authority, 2008; Sydney Catchment Authority, 2009). Different guidelines and standards apply to each part of the supply cycle as water passes from watershed waterways into reservoirs, and then into the distribution system or downstream rivers (Sydney Catchment Authority, 2007; Sydney Catchment Authority, 2008; Sydney Catchment Authority, 2009). The monitoring of the CB and their associated toxins in catchment and reservoir is following Australian and New Zealand Environment and Conservation Council (ANZECC) water quality guidelines (Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, 2000) and Australian Drinking Water Guidelines (Australian National Health and Medical Research Council, 2004), respectively and all analysis is completed to NATA accreditation (SI 8.C Table SI 8.C.1). Cases where total CB biovolume exceeds 4 mm^3/L, or where a visible algal scum persists in the area, are also classified as an incident in recreational waters (Sydney Catchment Authority, 2007). Such events trigger additional monitoring including analysis for the presence of algal toxins. Where potentially toxin producing species are detected above threshold values, the concentration of toxin must be measured. The following guidelines apply for drinking water reservoirs: (1) the maximum limit for potentially toxin producing cells is 6500 cells/mL, and (2) the maximum limit for toxicity is 1.3 µg/L Microcystin-LR (MC-LR) equivalent. The following recreational guidelines apply for lakes that do not directly supply water for treatment and for all bulk water transfers and releases: (1) the maximum limit for potentially toxin producing cells is 50000 cells/mL, (2) the maximum limit for CB biovolume is 4 mm^3/L, (3) the maximum limit for toxicity is 10 µg/L MC-LR equivalent (Sydney Catchment Authority, 2007).

The SCA monitoring program is defined by two criteria: (1) Whether the storage is online or not (whether another storage is a barrier to drinking water supply) and (2) the long term historical risk of CB issues in the water body. SCA surveillance program includes fortnightly CB monitoring in Dam reservoirs (e.g. Lake Burragorang), and monthly at other lakes from June to September. As the risk of CB is greater in summer months, seasonal sampling is conducted weekly from October to May. In addition to routine water quality monitoring, additional samples are collected under targeted, investigative and event-based monitoring at various locations. CB cell counts are only undertaken at upstream sites when chlorophyll*a* (Chl*a*) is greater than 5 µg/L (Sydney Catchment Authority, 2007).

The SCA recorded a major CB bloom on August 2007 due to significant inflows into Lake Burragorang (June 2007) (Sydney Catchment Authority, 2008; SA Water 2007). This incident did not result in any significant deterioration of water supplied for treatment but required careful management to maintain the quality of raw water to protect public health. The SCA developed "contingency plans" to ensure continued supply in the case of potential deterioration of water quality. This bloom continued to October/November 2007, The SCA invited AWQC biology research team (Drs. Mike Burch, Peter Hobson and Rob I. Daly) to undertake a real-time monitoring task to understand the nature and distribution of CB in Lake Burragorang. CB mapping was conducted on Lake Burragorang (Warragamba Dam) using YSI-BGA PC probe and YSI 6600V2 multi-probe system (SA Water 2007). Mean concentration of CB recorded by YSI-BGA probe between 0-10 m depths from dam wall are presented in SI 8.C Figure SI 8.C.2. Also in 11 points over the reservoir the presence of CB up to the depth of 40m was monitored by the YSA probe (SI 8.C Figure SI 8.C.3). The presence of CB in high numbers has been detected in depth of 15 m. At that time, the SCA was drawing water from 35 metres below the surface of Lake Burragorang to prevent the CB flow into the treatment facilities (Sydney Catchment Authority, 2008).

The online monitoring helped SCA to better understand (a) the CB dynamics in the reservoir (Sydney Catchment Authority, 2007) and (b) the limitations of the probe. SCA have been facing some issues with the application of multi-probe systems mainly due to calibration of the optic probes. The SCA issues with these probes are very similar to the issues ÉPM team have been facing while using the probes (Zamyadi et al. 2008). These issues and some other bias involved

with the *in vivo* probe measurements were discussed during this visit. The SCA experts‟ comments with regards to the need for modification of *in vivo* measurement techniques are valid.

F.2 New South Wales (NSW) Office of Water

The major river systems in NSW managed by NSW Office of Water includes two rivers (1) Darling River which is the Australia‟s longest river (2735 Km) and it is the major tributary of (2) the Murray River (2375 Km) (SI 8.D Figure SI 8.D.1). The NSW Algal Management Strategy is administered by the NSW State Algal Advisory Group and the nine Regional Algal Coordinating Committees (RACC) (SI 8.D Figure 8.D.2). The State Algal Advisory Group provides the over arching policy advice and framework for the management of fresh water and marine blooms (Detailed information on NSW administrative plan can be found on: http://www.water.nsw.gov.au/Water-Management/Water-quality/Algal-information/default.aspx).

Eutrophication is one of the main causes of toxic CB blooms in Murray-Darling River system, the other being regulation of these rivers and low flows in drought conditions. Eutrophication of this river system is enhanced by fertilizers movement from agricultural land and lawns, erosion of soil containing nutrients, sewage treatment plant discharges and urban runoff. Algal alerts are issued by RACC who are responsible for local management of algal blooms. There are three alert levels for recreational water use in NSW adapted to the local needs (these information are accessible to the public via www.water.nsw.gov.au):

- Red: These alert levels represent 'bloom' conditions. Red alerts are declared and public alerts issued when algal cell numbers exceed the threshold values identified in the Guidelines for Managing Risk in Recreational Waters published by the Australian National Health and Medical Research Council (2008). The water will appear green and may have strong, musty or organically polluted odours. CB may be visible as clumps or as scums. All CB 'blooms' should be considered to be toxic to humans and animals, and the water should not be used for potable water supply (without treatment), stock watering, or for recreation. There is also information provided for farm-dam management or irrigation and tourism during algal blooms on (SI 8.D Table SI 8.D.1).
- Amber: CB may be multiplying in numbers. The water may have a green tinge and musty or organic taste and odour. The water should be considered as unsuitable for potable use

without treatment. The water may also be unsuitable for stock watering. The water remains suitable for recreational use.

- Green: CB are first detected in the water at low amounts, possibly signalling the early stages of the development of a bloom. At these concentrations, the CB do not pose a threat to recreational, stock or domestic use.

Public alerts are not issued for Green and Amber alerts, although water utilities, landholders with livestock and other stakeholders are advised as necessary. Website (http://www.water.nsw.gov.au/) of NSW Office of Water provides a weekly updated public report based on the most recent algal data available from the NSW Office of Water laboratory and other sources. Red, Amber and Green alert data are collated by the RACC from across NSW and mapped for these updates. CB blooms may however be present elsewhere but not reported to the RACCs. The report also does not contain data from water storages managed by Water Supply Authorities where there is no public access. An example, from late April 2010, when a CB bloom at Amber alert levels dominated by *Anabaena circinalis* was reported throughout much of the NSW section of the Murray River (Upper and mid Murray River) is shown in SI 8.D Figure SI 8.D.3. The Murray River had been on Red alert for much of February and March 2010, prior to this.

Major bloom forming CB in NSW are *Microcystis aeruginosa* (MC), *Anabaena circinalis* (saxitoxins (STXs)) and *Cylindrospermopsis raciborskii* (cylindrospermopsin (CYN)). *Cylindrospermopsis sp.* bloom events are less common in the Murray-Darling basin while the other species are common across NSW. Current algal sampling for microscopic enumeration in NSW is (1) monthly in winter, (2) fortnightly in summer if no bloom and (3) weekly to bi-weekly when blooms, depending on water body and risk to the public. From spring 2010, field ELISA strips will be used for MC and STX detection in case of bloom events.

The NSW Office of Water have been trialling the use of YSI *in vivo* PC and Chl*a* probes as initial indicators of cyanobacterial bloom on limited basis, to determine the usefulness of these probes in remote field applications. The sampling team takes eight samples of one Litre from near the surface of the water body (maximum depth of 25cm). Each sampling point is approximately 15 m apart from other points. All 8 samples are mixed and the probe measurements then recorded over 3-5 minutes (data logging time every 10 seconds) for the

mixed samples. The detailed analysis of these data is currently ongoing under the supervision of Dr. Lee Bowling. Based on the preliminary results, high natural water turbidity (≥ 100NTU) has caused the probe to falsely detect CB. These results are coherent with laboratory validation of PC probes using different turbidity values (Zamyadi et al. 2008). Furthermore, it was found that raw probe readings, Relative Fluorescence Ratios (RFU), provide a better correlation with estimated cell biovolume from cell counts rather than probe readings in "cells/mL". The alert level (Red, Amber or Green) is determined using the total cyanobacterial biovolume of detected CB (SI 8.D Table SI 8.D.1). However, Dr. Lee Bowling"s team has come to the conclusion that the cellular biovolume of individual cyanobacterial species varies in time/space up to several times for the same species. This intra-species biovolume variation is a major challenge when defining biovolume-RFU alert levels.

NSW Office of Water is planning to equip its field monitoring teams with 6 new HydroLab / Turner Designs multi system probes that include PC and Chl*a* probes. These probes will be used for management purposes to monitor the changes in algal Chl*a* and PC as indicators of possible blooms, and provide early warnings of possible Red alerts. However any probe detection of high PC concentrations will be verified by microscopic enumeration. Experts of the NSW Office of Water believe that *in vivo* PC and Chl*a* probes may provide valuable data for high frequency monitoring of the changes in algal community. However, more historic data are required to establish a relationship between probe readings and biovolume/toxin production potential of present CB species. Dr. Lee Bowling (NSW Office of Water), Prof. Michèle Prévost, Arash Zamyadi (Ph.D. Student) and Sisi Zaho (M.Sc.) from ÉPM, and Sylvie Blais (MDDEP) are also involved in a mutual project on analysis of historic data of the Missisquoi Bay in Quebec.

F.3 South Australia Water (SA Water) Corporation and United Water/Veolia Water

The major river system in South Australia (SA) includes the Murray River flow in to the SA territory from NSW-Victoria border (SI 8.E Figure SI 8.E.1). Since the September 2007 severe drought situation in SA, SA Water has implemented a special project to provide a pre-emptive monitoring strategy for the River Murray through focused surveys of the river channel (Mosisch, 2008). The project is designed to provide a practical early warning system for drought related water quality issues along the SA stretches of the River Murray. More frequent toxic cyanobacterial bloom events were considered one of the main water quality issues during

drought season. Despite the recent rainfalls (Fall 2010), SA Water will follow the same monitoring plan not only for management consideration but also these frequent samplings provide SA water authorities and researchers an inclusive historic data.

The routine detection of CB toxins in source water raises several monitoring and treatment challenges. Moreover, the relation between the number of CB present in water and the probability of the presence of cyanotoxins is complex. High concentrations of CB bloom (field monitoring using PC *in vivo* probes) serve as a signal of a higher probability for toxins occurrence in water sources.

SA Water CB monitoring plan includes two main components (Mosisch, 2008):

1. Routine monitoring – enhanced River Murray routine monitoring program
2. Project-based – focused surveys and sampling; covered spring/summer and event based

1. To ensure detection of any bloom that may emerge, SA Water has implemented the following enhanced routine monitoring of the River Murray:

- 26 locations sampled weekly, including locations specifically focused on drinking water intakes. The main focus is to prevent the flow of CB cells into treatment facilities.
- Weekly routine algae, taste and odour (2-methylisoborneol (MIB) and Geosmin)
- In case of major blooms, toxin analyses/additional MIB and Geosmin

2. SA Water Team is providing a pre-emptive monitoring strategy for the River Murray through focused surveys of the river. Immediate on-the-ground assessment, tracking and early warning of potential water quality challenges (in particular CB blooms) is provided, to allow implementation of appropriate management/operational actions. SA Water River Murray monitoring field team is:

- Equipped with water quality probes including CB sensors (YSI *in vivo* PC probe and YSI multi-probe system) to monitor temporal and spatial changes in CB biomass in situ to indicate the possible onset of a bloom, thus enabling a quick operational response;
- Equipped with full microscopy capability for algal identification and enumeration in the field;

- Equipped with rapid toxicity test kits for STX and MC;
- Ground-truthing of aerial photography of the river (Mosisch, 2008).

The most common CB in the River Murray are the *Anabaena circinalis* and *Cylindrospermopsis raciborskii*. STX is the major toxin detected with *Anabaena circinalis* blooms in SA. More frequent toxic *Cylindrospermopsis sp.* bloom events have been recorded in last 5 years over Murray River (Mosisch, 2008). The subtropical species *Cylindrospermopsis raciborskii* has become more prevalent in the river. This cyanobacterium is more difficult to detect as it does not form a surface scum, and is distributed through the upper water column. It is not known to produce taste and odour compounds, but toxic bloom events have been observed over the river with production of CYN.

SA Water Morgan drinking water treatment plant (DWTP) (Murray River – Conventional filtration process) and Myponga DWTP (Myponga reservoir - Dissolved Air Flotation (DAF)) were also visited. These two DWTP like many other DWTP in SA are constantly facing the toxic CB issues during summer time. In the past they had problems with the accumulation of CB cell inside the plant and they were forced to seize the drinking water production for plant maintenance (filter cleaning, treatment adjustment by using different reactive, etc.). However the enhanced SA Water monitoring over the River and the reservoirs in recent years has helped them to prevent the flow of cells into the plants. Online CB monitoring using the *in vivo* probes is an essential part of CB related issues management in SA.

Because of water scarcity situation in Australia and particularly in SA water reuse is an important practice in providing irrigation water. Bolivar wastewater treatment facility is one of the Adelaide sewage treatment plants operated by United Water/Veolia Water. The treated sewage is processed through a DAF process, chlorination phase and then used for irrigation of plant distanced for human and animal consumption. However CB blooms in sewage treatment open lagoons is very common during the summer. Human exposure to cyanotoxins by consumption of plants irrigated with cyanotoxin contaminated recycled sewage is a limiting factor in water reuse in these conditions (Merel et al., 2010; Hoger, 2003; Newcombe, 2009). Furthermore high CB cell number will reduce efficacy of DAF process and augment the risk of DBP formation in chlorination process.

To reduce the risks associated with toxic CB the Research and Development (R&D) team of United Water is conducting tests for in situ CB monitoring on treated sewage from the open lagoons. Chl*a* in situ absorbance spectrometry is ongoing using Jaz Spectrometer Module (Ocean Optics) and s::can spectrophotometer (DCM process control). Chl*a* absorption spectrum has two wavelength peaks, 410-430 and 660-665 nm. The absorption wavelength peak at 410-430 nm is overlapping with absorbance peak of other algae pigments including red algae. To prevent the measurement bias due to this overlap, absorbance peak of 660-665 nm has been selected for the in situ monitoring. During the bloom season Jaz make measurements every hour and s::can every 2 minutes. Grab samples are taken 3 days per week for Chl*a* and PC extraction. Pigment extraction of samples from the last bloom season is still in process.

F.4 South East Queensland Water (Seqwater) and Australian Rivers Institute Griffith University

Samsonvale, Somerset and Wivenhoe are the three major lake/reservoirs owned by Seqwater in south east Queensland. Water stored within the reservoirs is used for a variety of purposes including potable use (Brisbane city and the Gold Coast), agriculture including irrigation and stock watering, industrial use such as power stations, and for recreation.

The prospect of increased occurrences of toxic CB in the reservoirs from drought prompted Seqwater to prepare the first version of its practical guideline in 2006 titled “Toxic Cyanobacteria Risk Assessment: Reservoir Vulnerability and Water Use Best Practice” (Orr and Schneider, 2006). This report assesses the risks to users from using or contacting water containing CB. It suggests guidelines to enable water users to assess the risk, and to make informed decisions about the taking and using of water (SI 8.F Table SI 8.F.1). The information presented in this report is drawn from a wide body of published scientific research, governmental guidelines, and is completed by data that is site–specific to Seqwater reservoirs, and for local water users. In preparation of this guideline new guidelines have been derived (by following standard methodologies and consulting leading researchers) where the existing guidelines are incomplete, and alternative guidelines have been provided where existing guidelines are inconsistent with site–specific considerations. This practical guideline is a good example of adapting the national and international guide books to site–specific problems: “The Seqwater practical guideline provides options for implementing and managing guidelines on a site–specific

basis to allow flexibility based on water use categories" (Orr and Schneider, 2006). Even though the principles of the 2006 guideline are still valid, Seqwater is now developing a formal "cyanobacterial management plan" which covers drinking water, recreational exposure and water quality to replace the 2006 document. New document is based on the Australian Drinking Water Guidelines, The Australian Guidelines for Managing Risk in Recreational Water; and the Australian and New Zealand Guidelines for Marine and Fresh Water Annual Seqwater R&D workshop (A. Zamyadi participated in the 2010 workshop on June 3rd and also presented a comprehensive seminar on the CB related research activities in ÉPM) provides a unique opportunity for the R&D team to adopt their activities to the novel scientific achievements and the new operational issues.

Seqwater reservoirs have a long history of CBl blooms. The dominant toxic species in reservoirs are *Cylindrospermopsis raciborskii* (CYN), although there are records of *Microcystis aeruginosa* (MC), *Anabaena circinalis* (STXs). Other potentially toxic species recorded in Seqwater reservoirs are *Anabaena bergii*, *Aphanizomenon ovalisporum* and *Rhaphidiopsis mediterranae* all of which potentially produce CYN in Australia.

Controlling CB blooms in reservoirs is a long-term process requiring a range of watershed and water quality management strategies that include managing nutrient inflows and reservoir hydrology. Predicting bloom occurrence is very difficult and is subject to pronounced uncertainty. Long term monitoring of limnological data can help the understanding of what drives CB growth and helps reduce the uncertainty associated with prediction. Seqwater in cooperation with Australian Rivers Institute is developing an index for predicting the reservoirs vulnerability to the CB blooms (Leighab et al. 2010).

Since 2008 the Lake Wivenhoe Integrated Wireless Sensor Network has been used to monitor processes that affect water quality at both a high spatial and temporal frequency (Dunbabin et al. 2009). The wireless sensor network includes 45 solar powered water-based nodes equipped with Fleck™ water temperature sensors, two YSI solar powered platforms with satellite connection equipped with YSI multi-probe system for water profile measurements (including PC probe) and a solar powered robotic boat as a mobile sensor platform. On June 4th 2010, a one day field trip was organized by Dr. James Udy of Seqwater to visit the Lake Wivenhoe Integrated Wireless Sensor Network. The visit included a boat trip over the Lake for inspection of the temperature

sensors and the YSI platforms, and discussions on probes calibration, application, biases and interpretation of the in situ data. In the short term real-time feedback to the reservoir and treatment plant operators can be used to modify the operational strategy based on in situ water quality data. The high resolution environmental data can also be applied in a longer term to improve the understanding of environmental factors that influence water quality in a water body and augment the accuracy of predictive models (Dunbabin et al. 2009).

F.5 Melbourne Water and Royal Melbourne Institute of Technology (RMIT)

The visit to Melbourne included (1) a meeting with the Melbourne Water R&D manager and process engineer on topics related to toxic CB removal in Australia and Canada, (2) an all exclusive visit to the Western Treatment Plant (WTP), and (3) a meeting with Dr. Thang Nguyen from Royal Melbourne Institute of Technology (RMIT).

WTP is located to the south-west of the Werribee Township (West of Melbourne) and receives around 47% (150 - 175 GL/y or 400 – 450 ML/d) of Melbourne‟s sewage for treatment. Sewage inflows at WTP have a high industrial component (McFarlane and McLennan, 2008). The treatment plant uses an activated sludge treatment process followed by 25 days retention in lagoons to produce Class C recycled water (SI 8.G Table SI 8.G.1). This water then undergoes Ultra Violet (UV) treatment followed by chlorination (typical free Cl_2 CT is around 30 mg.min/L) to produce Class A recycled water (SI 8.G Table SI 8.G.1) (McFarlane and McLennan, 2008). Class A recycled water is provided to: (1) onsite environmental lagoons and salinity management activities at WTP, (2) the Werribee Agriculture Group (WAG) for on-site irrigation for agriculture (mainly stock), (3) Southern Rural Water (SRW) for the Werribee Irrigation District (WID) scheme and the Werribee Tourist Precinct scheme, and to City West Water (CWW) for McKillop College and the Werribee Technology Precinct.

One of the main risk to recycled water within the main lagoon system (before Class C water production) is from a CB bloom (typically *Microcystis*), which can occur in the summer/autumn period as a result of increased sunlight, higher temperatures and generally lower plant inflows. The occurrence of CB blooms compromises the ability to meet the requirements for Class A recycled water, in particular related to the following two issues: (1) cyanotoxins which have been linked to skin irritations, allergic reactions and other health issues due to human exposure to these toxins by consumption of plants irrigated with cyanotoxins contaminated recycled sewage

(Merel et al. 2010; Hoger, 2003; Newcombe 2009); (2) CB blooms increase turbidity in the water which interferes with the effective functioning of the UV and chlorine disinfection processes. The supply of Class A recycled water has to be ceased if CB levels in Class C water reach 50000 cells/mL of *Microcystis* (equivalent of 10µg/L MC which is a conservative estimate but the recycled water will b used by WAG, SRW, WID and CWW) (McFarlane and McLennan, 2008).

The temporal variation of CB blooms made the in situ high frequency monitoring of the CB before disinfection process an operational necessity. Therefore Melbourne Water started the application of a bbe Moldaenke GmbH AlgaeOnlineAnalyser (AOA) as a part of routine operation procedure for Class C monitoring before UV disinfection. The probe is used to monitor the CB presence in water and in the case of CB peaks water samples are send for microscopic enumeration for better validation. This is an example of application of in situ CB monitoring in treatment plant operation. Currently, where a lagoon is affected by CB its entire throughput is discharged to Port Phillip Bay (Victoria, Australia).

Melbourne water is currently facing two issues with CB monitoring and oxidation:

(1) bbe AOA probe CB measurement is biased by high turbidity and presence of other algae in Class C water. This interference has been observed with other commercially available in situ probes (Zamyadi et al. 2009). Melbourne Water in cooperation with Dr. Thong Nguyen (RMIT) is conducting a research project to verify the sources of interferences and introduce possible solutions/modifications/correction factors.

(2) CB cell numbers are generally higher after Cl_2 oxidation than Class C water. Melbourne water engineers suspect this is because the chlorine and pumps have broken up some of the colonies so more cells is identified in enumeration. Results from a recent study on oxidation of CB cell showed that the exposure to the chlorine with a CT of 30 mg.min/L was enough to damage cell membrane and release the cell-bound material including toxins (Zamyadi et al. 2010). The challenge is to make sure that the released cell-bound toxins are oxidised under these chlorination conditions.

F.6 Australian Water Quality Centre (AWQC)

The visit to the AWQC includes:

a) R&D work on validation and development of a new generation of *in vivo* PC-Chl*a* probes; in collaboration with AWQC Biology Research team and YSI Inc.

b) R&D work on oxidation of CB cells and their associated toxins focusing on oxidation reaction rate and DBP formation; in collaboration with AWQC Applied chemistry team and Sydney Water.

c) Active participation in AWQC CB interest group meetings and presentation of the research work and results during a knowledge sharing seminar for AWQC and SA Water experts.

d) Interviewing the AWQC research leaders on toxic CB monitoring, removal, and publication of national and international management guidelines.

a) The previous work helped to identify the major sources of interferences involved with the *in vivo* PC probes measurements: Chl*a* from eukaryotic algae and water turbidity (Zamyadi et al. 2009). It was concluded from laboratory and field experiences (Zamyadi et al. 2009; McQuaid, 2009) that (1) parallel PC and Chl*a* measurement is a pertinent way to reduce the bias, and (2) the probes raw measurements has to be presented in relation to the cells biovolume to have a realistic estimation of the field conditions. Consequently, two prototype probes had been developed with parallel PC and Chl*a* measurements:

- PC prototype 1 (PCT1): 590 nm as excitation and 685 nm as emission wavelengths
- Chl*a* prototype 1 (Chl*a*1): 470 nm as excitation and 685 nm as emission wavelengths
- PC prototype 2 (PCT2): 610 nm as excitation and 685 nm as emission wavelengths
- Chl*a* prototype 2 (Chl*a*2): the same as Chl*a*1

The bandpass of probes readings is 40 nm. In this new generation of probes the PC and Chl*a* light emission diodes and photodetectors are installed in one probe. This setup insures that parallel PC and Chl*a* measurements are conducted in the same water volume instantly. These probes were tested in laboratory suspension of *Microcystis flos-aquae*, *Anabaena circinalis*, *Aphanizomenon* (CB) and *Scenedesmus* (eukaryotic algae). A good linear correlation ($R^2 \geq 0.95$) was observed between the probes readings in RFU and cells biovolume of different CB species from microscopic counts. Also a correction factor using the Chl*a* readings for the PC

measurement is on development. *In vivo* PC and Chl*a* were measured in unicellular suspensions of *Scenedesmus* (Figure F.1a). The PC measurement in these suspensions is a false reading as *Scenedesmus* has no accessory pigment. Afterwards, *in vivo* PC and Chla were measured in mix-suspensions of *Scenedesmus* and *M. flos-aquae* (the *M. flos-aquae* cell numbers were constant) (Figure F.1b). The control experiment was the PC RFU measurement in unicellular suspension of *M. flos-aquae* with the same CB cell number. By deducting the false RFU reading in absence of CB from the RFU reading in presence of CB a very good corrected PC RFU is acquired (Figure F.1b). The PC RFU in mix-suspension of CB and *Scenedesmus* after the correction is equal to the PC RFU in unicellular *M. flos-aquae* suspension with the same CB cell numbers (the control). Final verifications on the accuracy of the correction procedure are ongoing. However, based on a confidentiality agreement with AWQC and YSI, the correction procedure cannot be published until the development of the new probe is completed.

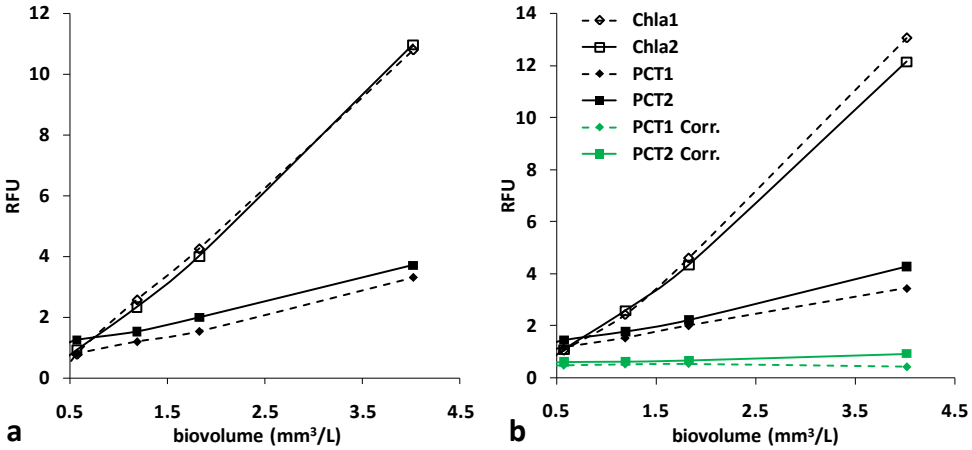


Figure F.1: Cells biovolume vs. *in vivo* probes readings (a) in unicellular suspensions of *Scenedesmus*, and (b) in mix-suspensions of *Scenedesmus* and *M. flos-aquae* and corrected PC RFU readings.

b) DWTPs across the world are looking for solutions to prevent the entrance of toxic CB cells into the treatment process. While preventive source monitoring is a management option but it is required to study the feasibility of pre-treatment techniques for the cells removal before the plant. Pre-oxidation using chlorine is a potential option. The objective of the R&D work on the oxidation of cells was to answer these questions: (1) Can chlorine exposure (expressed in CT)

predict toxin release for different CB species present at different densities (in environmental bloom samples) submitted to different initial chlorine dosages? And (2) what is the impact of pre-chlorination on the DBP formation? The resistance to chlorination of four potentially toxic CB species (*Anabaena circinalis*, *Microcystis aeruginosa*, *Aphanizomenon issatschenkoi*, and *Cylindrospermopsis raciborskii*) and their associated toxins (STX, MC, CYN) in water samples from Sydney Water was studied. Also the formation of DBP during the chlorination experiments was investigated. The analysis of these recent data is ongoing and a paper is under preparation based on these results (for submission to the Journal of water Research). A part of these results have been published in the Journal of Environmental Science and Technology (Zamyadi et al. 2010).

c) AWQC CB interest group is regrouping AWQC research and operation teams (including applied chemistry, biology research and operation teams) working on CB problematic. CB problematic is complex and conducting research on CB related issues requires well organized multidisciplinary teams. AWQC CB interest group meetings are great opportunities for AWQC experts to organise their activities and share the outcome of their research.

d) The scientists in AWQC played a leading role in preparation of the Australian CB management guideline (Newcombe et al. 2010) (Management Strategies for Cyanobacteria (Blue-Green Algae): A Guide for Water Utilities) and the international one (Newcombe, 2009) (International guidance manual for the management of toxic cyanobacteria). To prepare these valuable guidelines they have followed a four steps process:

(1) The understanding of the amplitude of the CB problem specific to the studied region.

(2) The understanding of the practical/management needs of the local individuals/organisations dealing with CB related issues. To prepare the Australian guideline a workshop was organised with the participation of Australian water authorities to document their needs. Also an international workshop was organised in South Africa to prioritize the topics for the international guideline. AWQC research leaders suggest that a survey with participation of local water authorities will be faster, more economics and the outcome will be similar. The topics considered in these guidelines consist of the action the water authorities need to take in watershed management, source protection, recreational water activities and drinking water treatment. Water reuse is a new topic that needs to be studied more in detail.

(3) The understanding of the scientific and engineering CB management techniques.

(4) The adaptation of the existing knowledge to the site specific needs for a practical guideline, with a revision period of at least five years.

F.7 Water Research Centre (WRC) University of New South Wales (UNSW)

The visit to the WRC at UNSW was planned in parallel with participation at the International Water Association (IWA) 2010 Young Water Professionals Conference organised by the WRC. A. Zamyadi presented a platform seminar during this conference. A. Zamyadi has been involved with the activities of the IWA YWP Committee (YWPC) since the conference and he has been selected by IWA board of directors as the 2nd Vice Chair of the IWA YWPC and liaison with the IWA Specialist Groups.

The visit to WRC included visiting the laboratories and a meeting on toxic CB monitoring. The rapid estimation of CB concentrations and their toxins in the field is a necessary activity for effective management of the risks associated with the presence of cyanotoxins for recreational activities, drinking water production and water reuse; however, the existing technologies are not capable of this. For example, the microscopic enumeration method cannot provide *in vivo* results and requires highly qualified personnel. Real time quantitative polymerase chain reaction (qPCR) is a promising technique but again requires skilled personnel and is not yet available as an online technology. In contrast, fluorescence spectroscopy can theoretically provide an in situ estimation of cell density as CB possess Chl*a* and PC and on line probes based on this technology exist (McQuaid et al. 2011; Gregor et al. 2007). However, major interferences have been identified with the *in vivo* measurement of PC and Chl*a* using the *in vivo* probes (Zamyadi et al. 2009). Hence, it is required to see if CB excrete other specific fluorescent substances for excitation-emission wavelength analysis.

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SI F.A. Interview form for industrial visits in Australia

Fonds de recherche sur la nature et les technologies
Québec

Partnership research program on cyanobacteria
International internship

ÉCOLE POLYTECHNIQUE MONTRÉAL

Validation of *in vivo* phycocyanin probes for rapid cyanobacterial estimation and their application for *in situ* detection and monitoring of algal blooms

Water Authority:

Interviewed individuals:

Name	Position	Affiliation	E-mail	Telephone & Fax	Address

Information on water body(ies) experiencing cyanobacterial blooms:

Name	**Type** (River, lake/pond, dam reservoir)

Physical-hydraulic characteristics (Depth: Min, Max, average; if river: flow; seasonal water temperature; water quality homogeneity in profile):

Climate data (Temperature: day-night seasonal average; wind; seasonal precipitation):

Lake/pound/reservoir stratification (information on nutrients concentration in water/sediments):

Watershed (type: agricultural, urban, forest, etc.; size):

Water usages (presence of drinking water intake; the treatment process at DWTP; population served; recreational water usages: swimming, fishing, etc.):

Information on cyanobacterial blooms:

Temporal bloom records (Bloom seasons; the frequency of blooms; historic data):

Cyanobacterial species (All species found: cyanobacteria and other algae; biovolume of detected species; toxin production potential of detected species; dominant species in bloom; percentage of dominant species to all species):

Cyanobacterial presence in water profile (depth profile):

Cyanotoxins associated with the blooms (Identified toxins; Max, Min & average toxins concentrations; toxin stability in water even after bloom dispersion):

Record of any significant toxins related impact on human/animal health:

Information on Probe application:

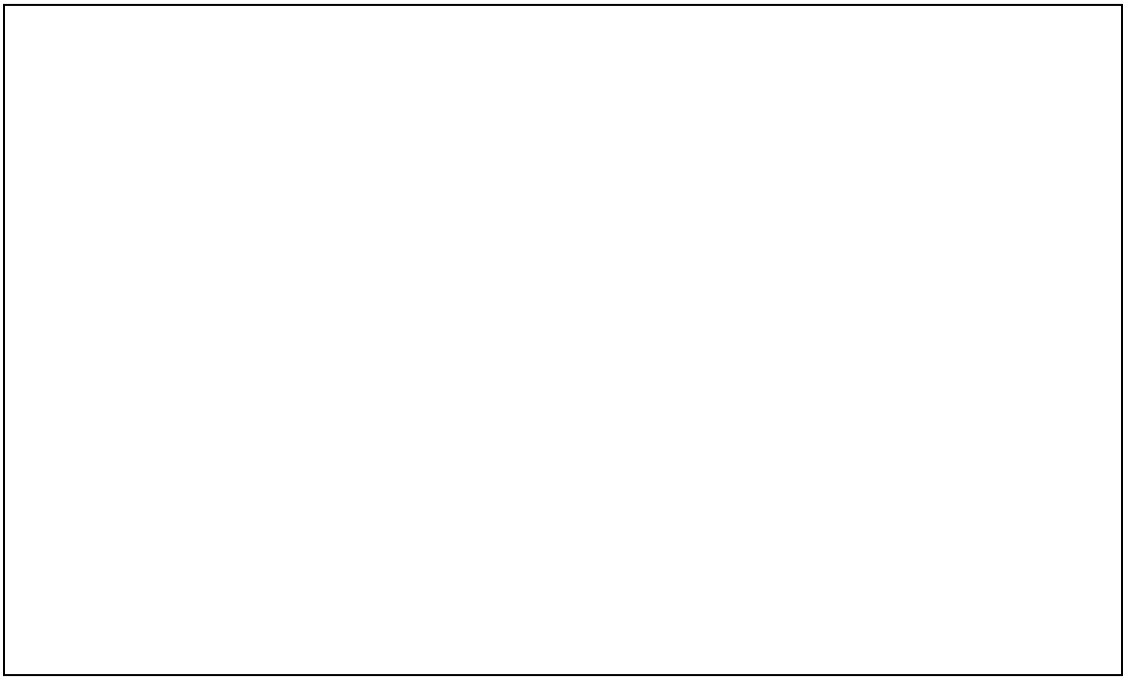
Monitoring strategy (monitoring method/instrument; monitoring frequency; sampling instrument):

For which purpose is the *in vivo* probe used:

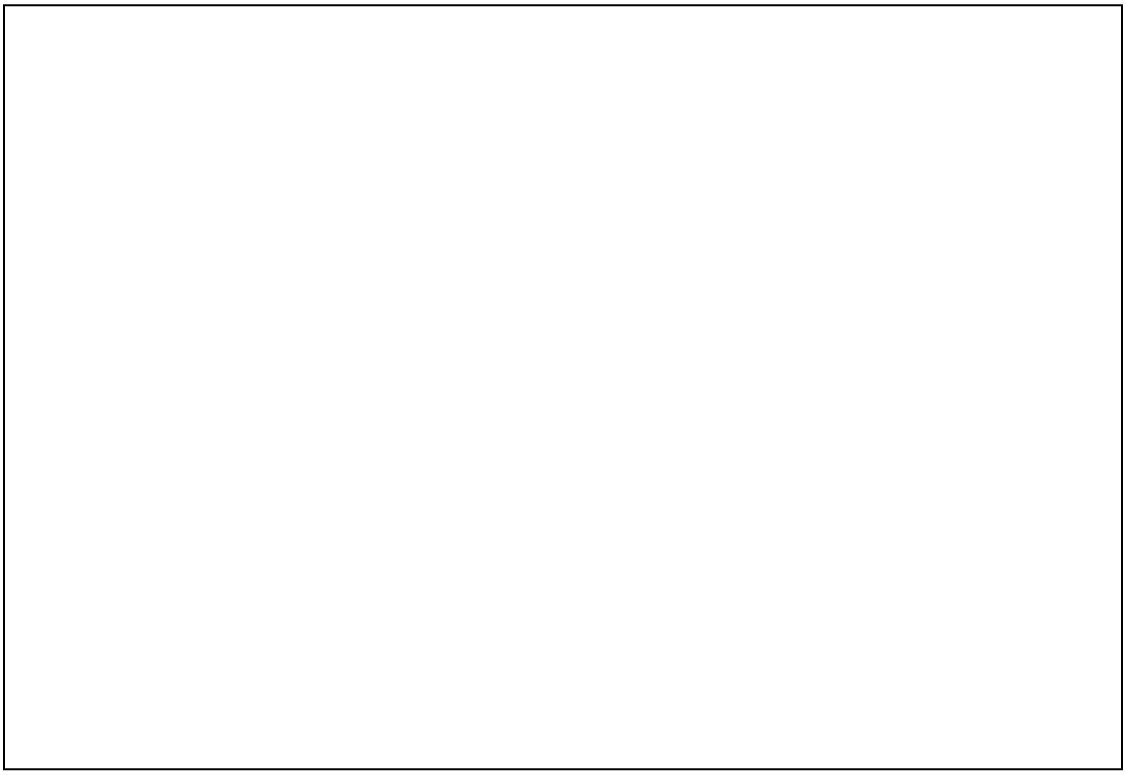
The reason behind selection of a particular probe/multichannel system (which water quality parameters are measured by the probe; why these parameters):

Probe calibration procedure:

Probe application procedure:



Correlation between the cyanobacteria (biovolume/*in vivo* probe measurements/taxonomic enumeration) and toxin presence:



Other observations/information & attached documents/maps etc.:

Interviewed by:

Name	Position	Affiliation	E-mail	date

SI F.B. Name and affiliation of interviewed individuals

Name and affiliation* of interviewed individuals are listed as follows:

Name	Position	Affiliation	Address
Dr. Bala Vigneswaran	Senior Water Quality Scientist	Sydney Catchment Authority	PO Box 323 Penrith NSW 2751, Australia Level 2, 311 High Street, Penrith NSW 2750, Australia
Shane D. Faulkner	Limnologist		
Dr. Lee Bowling	Principal Limnologist	NSW Office of Water	10 Valentine Avenue, PO Box 3720, Parramatta NSW, 2124, Australia
Dr. Thorsten Mosisch	Source Water Quality Manager, Water Quality & Integrated Management	SA Water	SA Water House 250 Victoria Square, Adelaide SA, 5000, Australia
Vladimir Tsymbal	Source monitoring officer		
Dr. Stéphanie Rinck-Pfeiffer	Research & Development Manager	United Water Veolia Water	180, Greenhill Road Parkside Adelaide SA, 5063, Australia
Dr. Robert May	Research Engineer		
Michael Nicholas	Research Engineer		
Dr. James Udy	Principal Scientist and Research manager	Seqwater and University of Queensland	Seqwater House 240, Margaret Street, Brisbane QLD, 4000, Australia
Dr. Philip Orr	Senior Research Scientist	Seqwater	
Dr Michele Burford	Principal Research Fellow	Australian Rivers Institute Griffith University	Griffith University, Nathan, Queensland 4111, Australia
Dr. Judy Blackbeard	Manager, Water Recycling Research	Melbourne Water	100, Wellington Parade, East Melbourne VIC, 3002, Australia
Danny Murphy	Process Engineer, Research & Technology		
Dr. Thang Nguyen	Research Fellow at School of Civil, Environmental & Chemical Engineering	Royal Melbourne Institute of Technology (RMIT)	City campus, Building:7, Level:2, Room:4 124 La Trobe Street Melbourne VIC, 3000, Australia
Dr. Gayle Newcombe	Senior scientist and Research team leader	Australian Water Quality Centre (AWQC)	SA Water House 250 Victoria Square, Adelaide SA, 5000, Australia
Dr. Mike Burch	Senior scientist and Research team leader		
Dr. Lionel Ho	Senior scientist		
Dr. Peter Baker	Senior scientist and Operation team leader		
Dr. Andrew Humpage	Senior scientist		
Dr. Rob I. Daly	Senior scientist		
Dr. Julie Culbert	Senior scientist and research officer		
Dr. Rita Henderson	Senior Research Associate	Water Research Centre (WRC) University of New South Wales (UNSW)	UNSW Water Research Centre School of Civil and Environmental Engineering University of New South Wales Sydney NSW 2052

* The six Australian water authorities/industries visited during this trip are: (1) Sydney Catchment Authority (SCA), (2) New South Wales (NSW) Office of Water, (3) South Australia Water (SA Water) Corporation, (4) United Water/Veolia Water, (5) South East Queensland Water (Seqwater), and (6) Melbourne Water. The four Australian research centres visited parallel or separate to the water authorities are: (1) Australian Water Quality Centre (AWQC), (2) Australian Rivers Institute Griffith University, (3) Royal Melbourne Institute of Technology (RMIT), and (4) Water Research Centre (WRC) University of New South Wales (UNSW).

SI F.C. Supplementary information from SCA

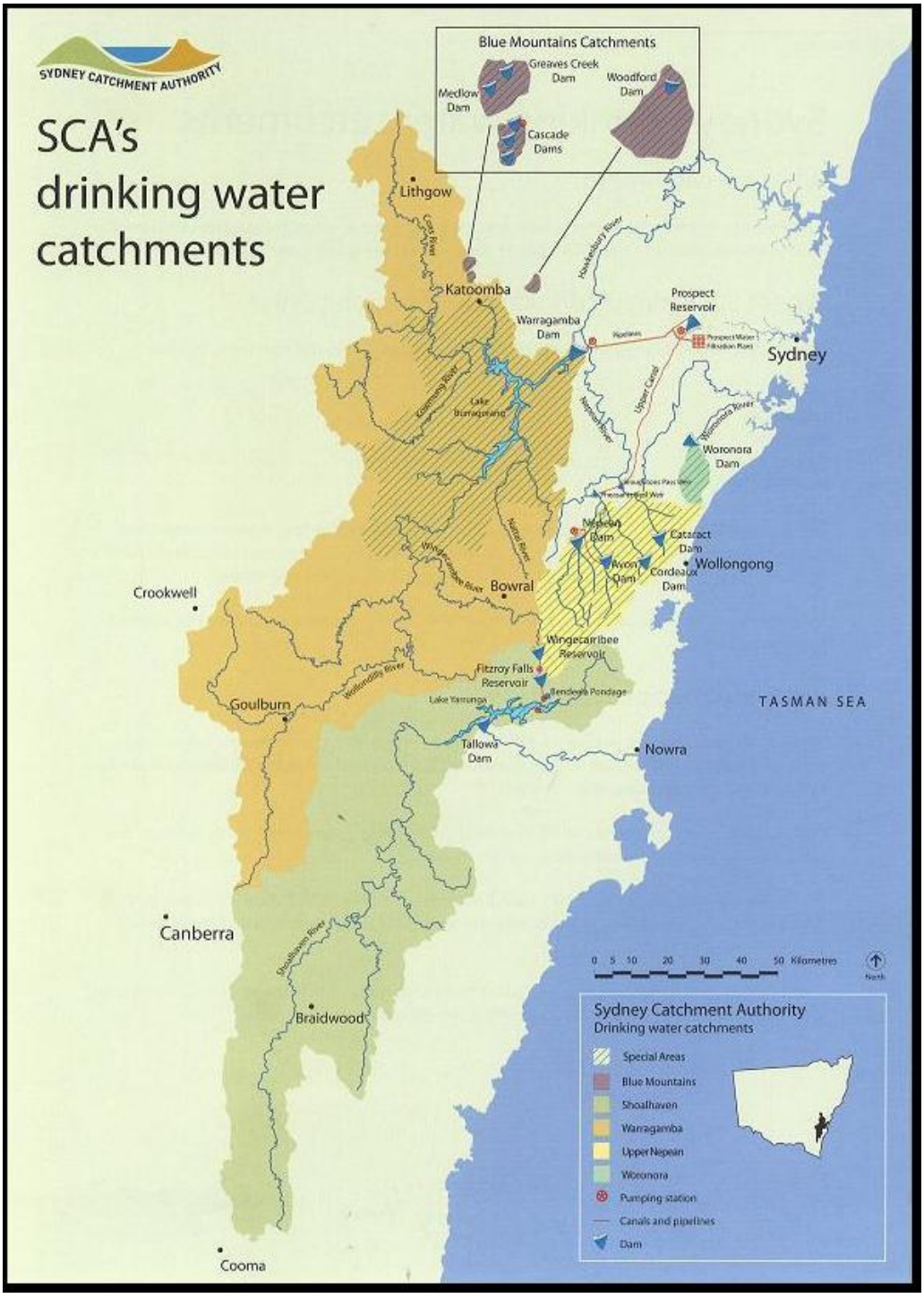


Figure SI F.C.1: Map of catchments managed by SCA (Adapted from: Adapted from Sydney`s drinking water catchments published by SCA)

Table SI F.C.1: Water quality guidelines for catchments and reservoirs (Sydney Catchment Authority, 2008; Sydney Catchment Authority, 2009)

Analyte (units)	Catchment	Reservoir
Chlorophyll-a (µg/L)	5	5
Algal ASU* (per mL)	n/a	2,000
Potentially toxin producing cyanobacteria (cells/mL)		
-recreational guideline	n/a	50,000
-drinking water guideline	n/a	6,500
Cyanobacteria biovolume (mm^3/L)	n/a	4

*ASU: Areal Standard Unit (a measure of cyanobacteria)

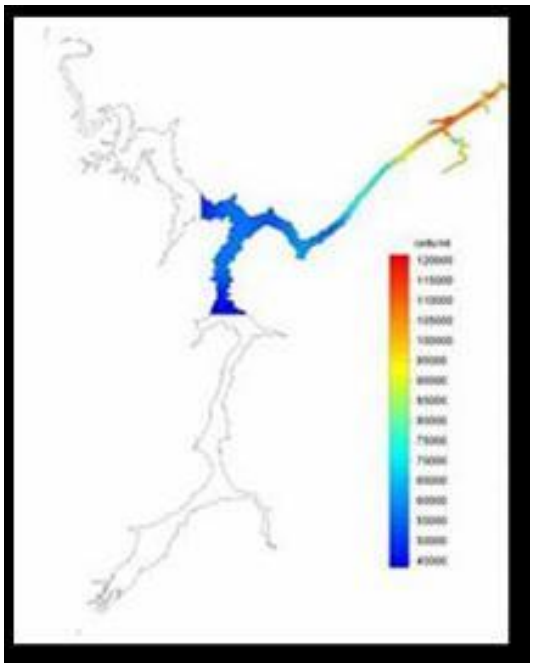


Figure SI F.C.2: Surface distribution of CB in Lake Burragorang, 19 September 2007 (Adapted from: Dr. Mike Burch, South Australia Water Research Development and Innovation Workshop 2008 presentations, University of Adelaide, Australia) (SA Water 2007)

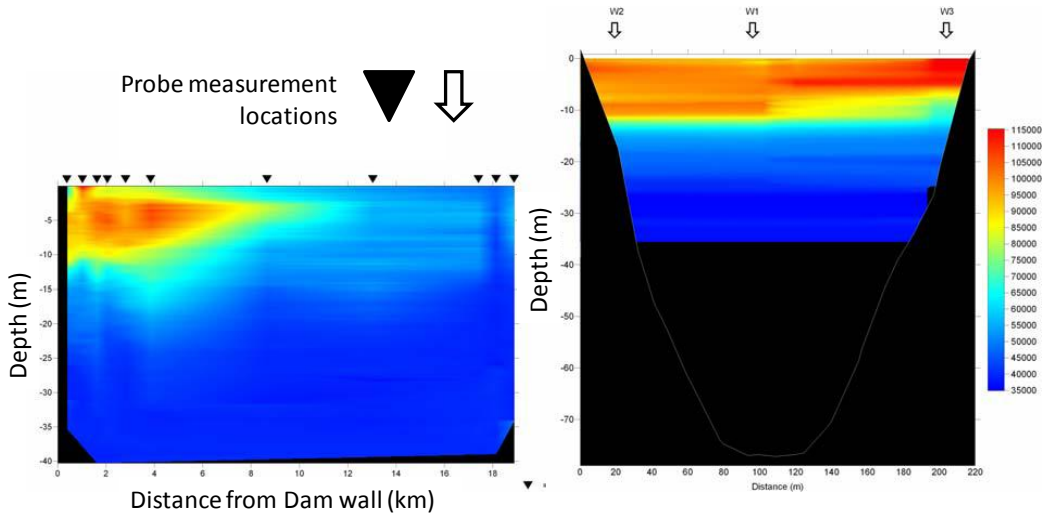


Figure SI F.C.3: Sub-surface distribution* of CB in Lake Burragorang, 19 September 2007 (Adapted from: Dr. Mike Burch, South Australia Water Research Development and Innovation Workshop 2008 presentations, University of Adelaide, Australia) (SA Water 2007)

* Water in deep Lake Burragorang tends to be warmer near the surface and colder at depth because surface water is warmed by the sun, particularly during summer months. Since cold water is denser, there is also a density gradient, with water temperature decreasing with depth in the lake. Often water separates into two distinct layers called the epilimnion (upper layer) and hypolimnion (lower layer), separated by a sharp temperature difference (called a thermocline) (Sydney Catchment Authority, 2007). These two water masses can have distinct characteristics (in addition to temperature and density) that affect water quality and living organisms (especially algae).

SI F.D. Supplementary information from NSW Office of Water

Figure SI F.D.1: Map of Darling-Murray rivers system (Adapted from: http://www.water.nsw.gov.au/Water-Management/Water-quality/Algal-information/default.aspx)

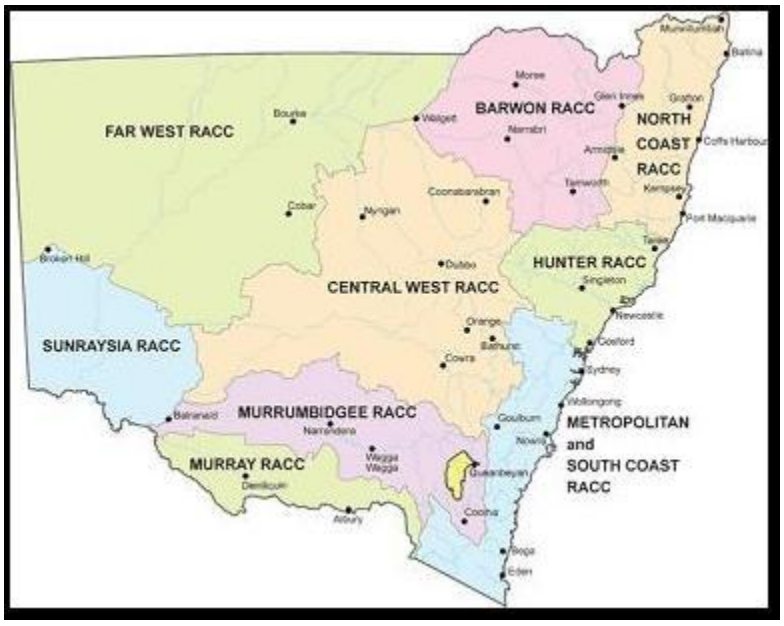


Figure SI F.D.2: Map of Regional Algal Coordinating Committees (RACC) in NSW (Adapted from: http://www.water.nsw.gov.au/Water-Management/Water-quality/Algal-information/default.aspx)

Table SI F.D.1: Alert levels based on biovolume (Adapted from: information discussed with Dr. Lee Bowling from NSW Office of Water))

NSW algal alert level	Cyanobacteria biovolume (mm^3/L)
Green	0.04
Amber	0.4
Red	4.0

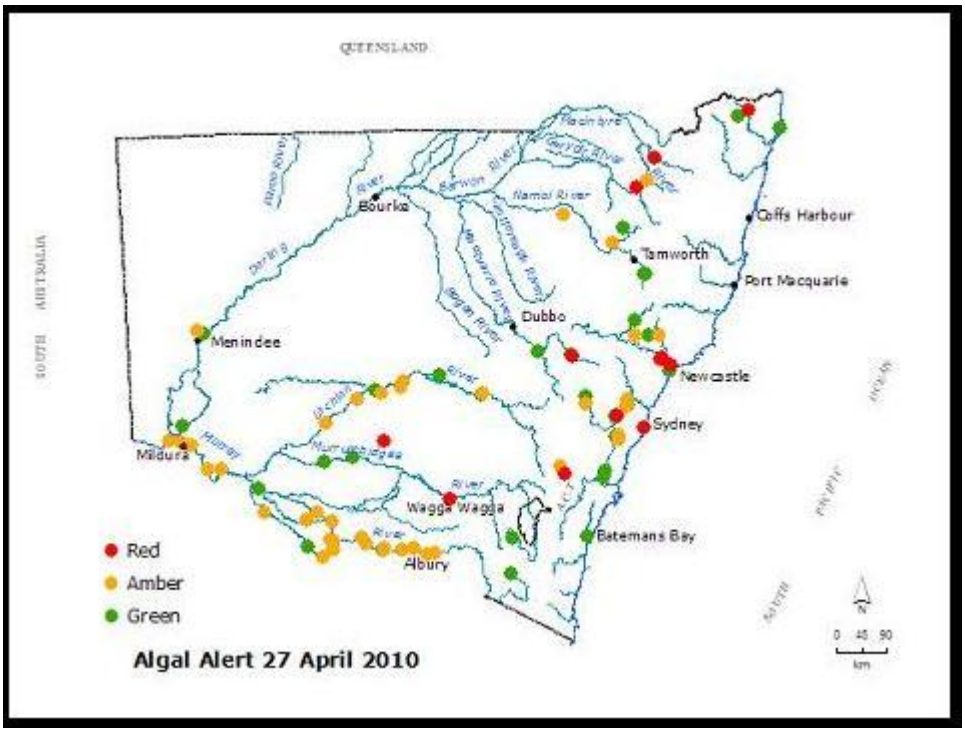


Figure SI F.D.3: Example of map of algal alerts in New South Wales (Locations identified below were experiencing algal blooms at the date of the report) (Adapted from: http://www.water.nsw.gov.au/Water-Management/Water-quality/Algal-information/default.aspx)

SI F.E. Supplementary information from SA Water

Figure SI F.E.1: Map of Murray River water distribution system in SA (Adapted from: SA Water Research Development and Innovation Workshop 2008 presentations, University of Adelaide, Australia) (Mosisch, 2008)

SI F.F. Supplementary information from Seqwater

Table SI F.F.1: Advisory levels derived in this report for the range of water use categories for which water sourced from Seqwater reservoirs is put (Adapted from: Orr and Schneider, 2006)

Advisory	Indicative cell or toxin concentration					
	M. aeruginosa cells mL^{-1}	MCYST-LR $\mu g\ L^{-1}$	*A. circinalis* cells mL^{-1}	STX $\mu g\ L^{-1}$	CYN $\mu g\ L^{-1}$	Total Cyanobacteria cells mL^{-1}
Potable Water	6,500	1.3	20,000	3	1.0	-
OHS (Aerosols)	13,000	3	20,000	3	10	-
Livestock (low value)	50,000	10	150,000	22	7	Scums
Livestock (high value)	10,000*	2.0	25,000*	3.8	1	Scums
Aerosols	13,000	2.6	20,000	3	10	
Recreation yellow – orange – red–	-	-	-	-	-	<20,000 >20,000 >100,000 (or scums)

MCYST-LR = Microcystin-LR toxicity equivalents; STX is saxitoxin toxicity equivalents; and CYN is cylindrospermopsin
* With the option for a lower value to apply to chickens, alpacas and horses

SI F.G. Supplementary information from Melbourne Water

Table SI F.G.1: Classes of reclaimed water and standards for biological treatment & pathogen reduction according to Australian Environmental Protection Agency Guidelines (Adapted from: McFarlane and McLennan, 2008).

Class	*Water quality objectives - medians unless specified*[1,2]	*Treatment processes*[3]	*Range of uses– uses include all lower class uses*
A	Indicative objectives • < 10 *E.coli* org/100 mL • Turbidity < 2 NTU[4] • < 10 / 5 mg/L BOD / SS • pH 6 – 9[5] • 1 mg/L Cl_2 residual (or equivalent disinfection)[6]	Tertiary and pathogen reduction[7] with sufficient log reductions to achieve: <10 *E.coli* per 100 mL; <1 helminth per litre; < 1 protozoa per 50 litres; & < 1 virus per 50 litres.	Urban (non-potable): with uncontrolled public access Agricultural: eg human food crops consumed raw Industrial: open systems with worker exposure potential
B	• <100 *E.coli* org/100 mL • pH 6 – 9[5] • < 20 / 30 mg/L BOD / SS[8]	Secondary and pathogen (including helminth reduction for cattle grazing) reduction[7]	Agricultural: eg dairy cattle grazing Industrial: eg washdown water
C	• <1000 *E.coli* org/100 mL • pH 6 – 9[5] • < 20 / 30 mg/L BOD / SS[8]	Secondary and pathogen reduction[7] (including helminth reduction for cattle grazing use schemes)	Urban (non-potable) with controlled public access Agricultural: eg human food crops cooked/processed, grazing/fodder for livestock Industrial: systems with no potential worker exposure
D	• <10000 *E.coli* org/100 mL • pH 6 – 9[5] • < 20 / 30 mg/L BOD / SS[8]	Secondary	Agricultural: non-food crops including instant turf, woodlots, flowers