Release of Intracellular Metabolites From Cyanobacteria During Oxidation Processes [Project #4406]

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PRINCIPAL INVESTIGATORS:
Eric C. Wert, Mei Mei Dong, Fernando L. Rosario-Ortiz, and Julie Korak

OBJECTIVES

The objective of this project was to determine the effect of common preoxidants used during drinking water treatment on the integrity of cyanobacteria cells, and to evaluate the subsequent release of toxic metabolites, odorous metabolites, and disinfection byproduct (DBP) precursors.

BACKGROUND

Drinking water utilities often begin the treatment process by using an oxidation process to meet different water quality objectives (e.g., disinfection, control of invasive species, oxidation of organic/inorganic contaminants). If cyanobacteria cells are not physically removed prior to oxidation, cell damage or lysis may result in the release of intracellular (or cell bound) organic matter (IOM) into the water supply. The IOM may contain algal toxins (e.g., microcystin, cylindrospermospin, anatoxin), taste and odor compounds (e.g., 2-methylisoborneol [MIB], geosmin), or disinfection byproduct precursors. Few studies have investigated the risk of releasing cell-bound IOM during oxidation processes including ozone, chlorine, chlorine dioxide, and chloramines.

APPROACH

Three cyanobacteria (i.e., *Microcystis aeruginosa* [MA], *Oscillatoria* sp. [OSC], and *Lyngbya* sp. [LYN]) were selected for the study based upon (1) occurrence in source water supplies, (2) availability of an axenic culture, (3) ability to produce odorous or toxic metabolites, and (4) cell morphology. These cyanobacteria were placed into a growth chamber under controlled light and temperature conditions. Cells were harvested around the late exponential or early stationary growth phase (28 days).

A purified cell suspension was used to evaluate cyanobacteria cell integrity and metabolite release in Colorado River water. In order to evaluate cell degradation, digital flow cytometry (FlowCAM) in combination with chlorophyll-a measurements provide an assessment...
of cyanobacteria cell damage and lysis after exposure to ozone, chlorine, chlorine dioxide, or chloramine. Digital and binary images were collected by the FlowCAM to provide qualitative information regarding cyanobacteria cell damage. The release of toxic (i.e., microcystin-LR [MC-LR]) or odorous metabolites (i.e., MIB, geosmin) was investigated after filtration of the damaged cyanobacteria cells.

DBP formation was evaluated using a standard of IOM extracted from each of the cyanobacteria species. IOM was extracted using a freeze-thaw/sonication/filtration process. Formation potential (FP) testing was conducting using chlorine and chloramines. FP testing with chlorine focused on the formation of total organic halogen (TOX), trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs), and trichloronitromethane (or chloropicrin). FP testing with chloramines focused on the formation of nitrosamines (including N-nitrosodimethylamine [NDMA]).

RESULTS/CONCLUSIONS

The digital flow cytometer provided a rapid method to obtain quantitative and qualitative information regarding cyanobacteria cell damage and lysis compared to conventional light microscopy. Cell damage was defined by the loss of chlorophyll-a or a decrease in the fluorescent particle concentration measured using the FlowCAM. Cell lysis was defined by the fragmentation of the unicellular or filamentous structure. Results showed that cyanobacteria cell damage occurred without complete lysis or fragmentation of the cell membrane under the conditions tested. During ozone and chlorine experiments, the unicellular MA was more susceptible to oxidation than the filamentous OSC and LYN. Digital and binary images provided some perspective regarding how the oxidants may be damaging the cell structure. In most cases, the oxidant appeared to penetrate the cell wall and oxidize or release chlorophyll-a and other metabolites without completely fragmenting the cell membrane.

The release of metabolites was measured after filtration of the damaged cyanobacteria cells: MA (200,000 cells/mL), OSC (2,800 cells/mL), and LYN (1,600 cells/mL). The DOC release after oxidation was less than 0.3 mg/L under the conditions tested. During the oxidation of 200,000 cells/mL of MA, release of intracellular MC-LR exceeded 1 µg/L during the lowest oxidant exposures (CT) tested: ozone (0 mg-min/L, dose below the ozone demand), chlorine (<40 mg-min/L), chlorine dioxide (<558 mg-min/L), and chloramine (<636 mg-min/L). As the CT increased, ozone, chlorine, and chloramine dioxide were able to oxidize the released MC-LR, which was expected based upon published kinetic information.

MIB and geosmin release were investigated after oxidation of OSC and LYN cells. Releases of MIB and geosmin exceeded their respective threshold odor values after oxidation by chlorine, chlorine dioxide, and chloramine. These oxidants have low reactivity with MIB and geosmin. When insufficient ozone residual was established (below or near demand), there was a measured increase in MIB and geosmin concentration. As ozone CT increased, hydroxyl radicals were able to oxidize the release MIB and geosmin, which was expected based upon published kinetic information.

DBP yields from IOM were identified for carbonaceous and nitrogenous species. FP testing showed that IOM has the potential to provide another source of DBP precursors. During FP testing with chlorine, most of the TOX was identified (62–78%) among THMs, HAAs, TCNM, and HANs. TOX is a surrogate for the overall formation of halogenated DBPs in a sample. During FP testing with 100 µg/L of bromide, the formation of different brominated
DBPs was observed, although overall mass yields of specific classes of DBPs were unchanged. During FP testing with chloramines, NDMA yields were identified between 10–52 ng/mg C depending on the source of the IOM (MA, OSC, or LYN). Other nitrosamines were also formed including \(N\)-nitrosodimethylamine, \(N\)-nitrosopyrrolidine, and \(N\)-nitrosopiperidine. The IOM was also analyzed for assimilable organic carbon (AOC). Results showed that >99% of the IOM extracted from OSC and LYN was present as AOC. Therefore, IOM may be removed during a biological treatment process.

APPLICATIONS/RECOMMENDATIONS

Utilities can use this research to better understand the impact of their pretreatment strategy on the risk of releasing toxic metabolites, odorous metabolites, and/or DBP precursors. Results from this study showed that low oxidant exposures can result in the release of cyanobacteria metabolites. Depending on the cell concentration, oxidant exposure, and the magnitude of DOC release, sufficient IOM concentrations may be released resulting in impacts on regulatory compliance (THMs and HAAs) or consumer confidence (MIB and geosmin). With respect to MC-LR, utilities using chloramines ahead of any physical treatment barrier are at the greatest risk for releasing and accumulating MC-LR within the treatment process. With respect to MIB and geosmin, utilities using chlorine, chlorine dioxide, or chloramines are at the greatest risk for releasing and accumulating MIB and geosmin within the treatment process. Ozonation has shown the ability to release metabolites. However, ozone reacts rapidly with MC-LR and hydroxyl radicals react with MIB and geosmin, which can minimize the effect of the metabolite release via cell damage. Overall, physical removal of cells is recommended before the primary disinfection step in a water treatment process to avoid the release of these metabolites.

FP testing identified the potential to form DBPs from IOM sources. During cyanobacteria cell testing, DOC releases did not exceed 0.3 mg/L based on the cell concentrations used in this study. Therefore, the magnitude of DOC release and subsequent chlorine or chloramine exposure must be taken into consideration when evaluating DBP risk. Furthermore, AOC results showed IOM from OSC and LYN to be largely biodegradable. This finding could indicate that cyanobacteria-derived organic matter released into the environment (perhaps during cell decay) may be biodegraded before entering the water treatment facility. It also implies that any released IOM may be removed (along with corresponding DBP precursors) through a biological treatment process. Therefore, the significance of IOM as a DBP precursor source may be very site specific depending on cyanobacteria specie, cell concentration, oxidation conditions, magnitude of DOC release, and subsequent chlorine or chloramine exposure.

RESEARCH PARTNER

Southern Nevada Water Authority